Epidemiology of Rotavirus in Southern Vietnam: Results of a Sentinel Surveillance From 2013 to 2018

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Epidemiology of Rotavirus in Southern Vietnam: Results of a Sentinel Surveillance From 2013 to 2018

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A Master's Thesis
Submitted to the Department of Global Health Security,
Division of Global Health Security Detection Program
and the Graduate School Public Health of Yonsei University
in partial fulfillment of the
requirements for the degree of
Master of Public Health

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December 2019

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December 2019

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to Professor Sangchul Yoon and Professor Joon Sup Yeom, my research supervisors. Thank you for all the instructions, ideas, and moral support in guiding me through this project. Your advice on both research as well as on my career has been invaluable.

I would like to thank my thesis committee member, Professor Hoon Sang Lee, for letting my defense be an enjoyable moment, and for your brilliant comments and suggestions.

Special thanks should also be given to my research mentor, Professor Ji-Man Kang, for your patience, enthusiastic guidance and encouragement in keeping my progress on schedule. I hope that there will be many more collaborations between us in the time to come.

I wish to thank various people in Vietnam for their contribution to this project. I would like to acknowledge the doctors, nurses, and patients at Children Hospital Number 1 in Ho Chi Minh City, for actively participated in the sentinel surveillance. Thanks to the laboratory technicians and surveillance staff at the Pasteur's Institute in Ho Chi Minh City, for their help in offering me the data resources. Thanks to the World Health Organization and the National Institute for Hygiene and Epidemiology for their support in organizing the Rotavirus sentinel surveillance in Southern Vietnam.

My grateful is also extended to the Korea International Cooperation Agency (KOICA) and the Graduate School of Public Health (Yonsei University), for giving me the wonderful opportunity to complete my Master's Degree in Korea. Thanks to Professor Myung Ken Lee, Professor Joshua Sir, Professor Moonsoo Yoon, for organizing such a great program. Thanks to all members of the Administration Office for their useful help during my master's course.

Finally, I wish to thank my beloved family. Words can not express how grateful I am to my parents, my brother, and sisters, for all of the sacrifices that you have made on my behalf. Your prayer for me was what sustained me thus far. I would also like to thank my beloved fiancé, Dr. Phan Thao Nguyen. Your devotion, unconditional love and support, sense of humor, optimism, and advice was more valuable than you could ever imagine.

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LIST OF ACRONYMS

Acute gastroenteritis	AGE
Case-fatality rate	CFR
Enzyme linked immunosorbent assay	ELISA
Intravenous fluids	IV
The Global Rotavirus Surveillance Network	GRSN
Oral rehydration solutions	ORS
Polymerase chain reaction	PCR
Center for Research and Production of Vaccines and Biologicals	POLYVAC
Vaccine effectiveness	VE
World Health Organization	WHO

Abstract in English

Rotavirus infects nearly every child by the age of 3–5 years and is the leading cause of severe, dehydrating diarrhea in children aged <5 years worldwide. In Vietnam, nearly haft of diarrhea-related deaths are accounted for Rotavirus; the country also had one of the highest Rotavirus hospitalization figures in South East Asia. The two Rotavirus vaccines (Rotarix and Rotateq) were licensed in the state and available in the private sector, as well as Rotavin-M1 - a local licensed vaccine. In 1998, the Rotavirus sentinel surveillance was initiated in Vietnam. This study aimed to describe the Rotavirus sentinel surveillance findings from 2013 to 2018, including epics profile, clinical features, and genotyping information among hospitalized children under five years of age. **Methods:** A hospital-based study was performed from the Rotavirus sentinel surveillance at Paediatric Hospital No.1 in Ho Chi Minh city, Vietnam. Data were collected from children between 0 and 59 months of age whose primary reason for hospitalization was acute watery diarrhea. Children were included in the vaccine effectiveness analysis (VE) if they were at least six months old at the time of notification. **Results:** During 2013-2018, of 5,179 acute diarrhea cases included in the study, Rotavirus was detected in 2,424 cases (46.80%), a downward trend was observed from 2013 (55.27%) to 2018 (43.54%). Dry season months (from November to April) witnessed a 2.4 times higher occurrence of Rotavirus positive cases compared to rainy season months (May to October) (OR=2.4, 95% CI: 2.14 - 2.692, $p<0.001$). The mortality rate was 0.04%. The typical symptoms associated with Rotavirus AGE were vomiting (present of vomiting, average max number per day, duration), dehydration status, present of fever, diarrhea (average max number per day) ($p<0.05$). Rotavirus AGE was more likely to be severe than the negative group ($p<0.001$). Of 1,107 Rotavirus positive cases had PCR isolated for genotyping, G3P[8] was the most common G-P combined genotype (43.18%), followed by G8P[8], G1P[8], G2P[4] (constituted 19.69%, 12.92%, and 12.83%, respectively). We observed an enormous genotyping shift from 2013 to 2018: G3P[8] became more frequent during 2014-2018, with the rise from 8.13% in 2013 to a peak of 60.65% in 2017, then decreased to 41.15% in 2018. In contrast,

G1P[8] considerably dropped from 69.92% in 2013 to no case in 2018. There also occurred a change in G8P[8] and G2P[4] prevalence. From no case in 2013, G8P[8] leaped to a peak of 47.21% in 2016 and was the predominant genotype at that year, before went down to 23.92% in 2018. G2P[4] showed a slight upward trend from 2013 to 2014 (from 15.45% to 37.41%), then dropped to 0.43% in 2016, before went up to 16.27% in 2018. The percentage of vaccinees among the study population was meager (3.84%). Among children ≥ 6 months of age, getting a completed schedule of Rotavirus vaccine in general, and the Rotarix vaccine in particular, had vaccine effectiveness against Rotavirus AGE at 82.34% (95% CI: 70.33%-89.49%), and 85.90% (95% CI: 74.10%-92.30%), respectively. Rotarix VE for G3P[8] was 88.35% (95% CI: 52.50%-97.14%). **Conclusions:** The findings in our study suggested that Rotavirus infection was still a significant cause of acute watery diarrhea among hospitalized children younger than five years old in Vietnam. There is a need to consider the recommended vaccine to use in the National Immunization Program in the dramatic genotyping shift situation. In addition to monitoring genotypes, whole genomic characterization of circulating Rotavirus strains before and after vaccine introduction will help to assess the vaccines' efficacy.

Chapter 1:

INTRODUCTION

1.1. BACKGROUND

Rotavirus infects nearly every child by the age of 3–5 years and is the leading cause of severe, dehydrating diarrhea in children under five years old worldwide. In low-income countries, the median age at the primary Rotavirus infection ranges from six to nine months (80% in infants less than one year of age) whereas in high-income countries, the first episode may be delayed until 2–5 years old, though the majority still happen in infancy (65% in infants under one year old) [1].

Estimates from 2013 determined that Vietnam had a total of 2,083 diarrhea-related deaths, of which 49.9% were due to Rotavirus. Rotavirus accounted for 3.1% total death of children under five years old [2]. The country also had one of the highest Rotavirus hospitalization figures in South East Asia [3].

In 1998, Vietnam launched Rotavirus surveillance under the auspices of the World Health Organization (WHO). During the period 2012–2015, a total of 8,889 children under five years of age were enrolled in the surveillance, in which Rotavirus was the most common pathogen (46.7%). A downtrend of G1P[8] prevalence was observed [4].

Rotavirus disease burden in Vietnam was estimated at approximately $3.1 million in direct medical costs, $685,000 in direct non-medical costs, and $1.5 million in indirect costs (Fischer et al., 2004) [5]. In 2009, data were collected from hospitalized children under five years old with symptoms of acute gastroenteritis (AGE) evaluated that costs of Rota-positive patients and Rota-negative patients were $217 and $158, respectively [6].

Vaccination offers the best protection for infants against severe diarrhea and death from Rotavirus infection, WHO recommends the routine use of Rotavirus vaccine in infants in all countries worldwide. Rotavirus vaccine was introduced in 101 countries by

2018. Global coverage was estimated at 35% [7].

In Vietnam, there are two WHO pre-qualified available (Rotarix and Rotateq), together with Rotavin-M1 - a local licensed vaccine manufactured by state-owned company POLYVAC (Center for Research and Production of Vaccines and Biologicals). The price of this Vietnamese-made vaccine is one-third the cost of an imported vaccine, at just $15 per dose. The second generation of Rotavin-M1 is evaluated on a clinical trial named Rotavin. The new vaccine is produced in the same way with Rotavin-M1 but more compatible with the cold chain currently in place in Vietnam, allowing for more accessible storage and transport [8].

Considering that Vietnam will introduce Rotavirus vaccines to the Expanded Program on Immunization soon, it is crucial and necessary to understand the epidemiology and genotyping diversity in pre-routine Rotavirus vaccines era. The disease surveillance system is one of the vital roles to provide this information supporting vaccine introduction decisions. Being a part of the National System, the Pasteur Institute in Ho Chi Minh City has been responsible for Rotavirus sentinel surveillance in Southern Vietnam, in coordinating with Children Hospital No. 1 in Ho Chi Minh City since 1998.

This study aimed to describe the Rotavirus sentinel surveillance findings from 2013 to 2018, including epics profile, clinical features, and genotyping information among hospitalized children under five years of age.

1.2. PURPOSE

1.2.1. Research questions

What is the epidemiology of Rotavirus acute gastroenteritis (AGE) and genetic characteristics of the strains circulating in Southern Vietnam?

1.2.2. Objectives

- General Objectives:

To determine the Rotavirus disease epics profile, genotyping information among children under five years of age in Southern Vietnam from 2013 to 2018.

- Specific Objectives:

+ To determine the prevalence, age, and geographic distribution, seasonality of Rotavirus disease among children aged <5 years in Southern Vietnam from 2013 to 2018.

+ To determine the clinical features of Rotavirus disease among children aged <5 years in Southern Vietnam from 2015 to 2018.

+ To determine the genotypic diversity of Rotavirus strains detected in children aged <5 years in Southern Vietnam from 2013-2018.

+ To determine the Rotavirus vaccine effectiveness in hospitalized children aged <5 years in Southern Vietnam from 2013-2018.

CHAPTER 2:

LITERATURE REVIEW

2.1. VIROLOGY OF ROTAVIRUS

2.1.1. History of the virus

Rotavirus is a virus that causes infection in the intestine. At the beginning of the 1970s, scientists who study bacteria, viruses, or parasites could only identify less than 30% of the number of diarrheal case in children. In 1973, thanks to an electron micrograph, Bishop and his colleagues successfully observed a virus particle in the intestinal tissue of diarrheal children [9]. In the same year, the name Rotavirus was suggested for its appearance under a microscope like a wheel, which means “Rota” in Latin [10].

2.1.2. Structure

As a genus of Reoviridae, the structure of Rotavirus consists of segmented viruses and double-stranded RNA genomes. Rotavirus has a massive particle that contains the viral genome of 11 of double-stranded RNA surrounded by a triple-layered protein capsid (an outer, inner capsid, and a core). Virus particle (viral protein [VPs]) is made up of 6 structural proteins and six nonstructural proteins. Only segment 11, which contains information for NSP5 and NSP6, encodes a single protein [11], [12].

Found in the internal capsid, VP6, which carries group-specific epitopes, is considered as the most highly represented structural protein [12], [13]. While three structural proteins (VP1, VP2, and VP3) and six nonstructural proteins (NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6) create the core of the virus, NSP4 creates a harmful substance for digestive system which called enterotoxin [11], [12], [14].

Forming the outer capsid shell and creating spikes that release through the outer capsid shell are respectively distinct functions of VP4 and VP7, which induce neutralizing antibody and also identify viral serotypes [11].

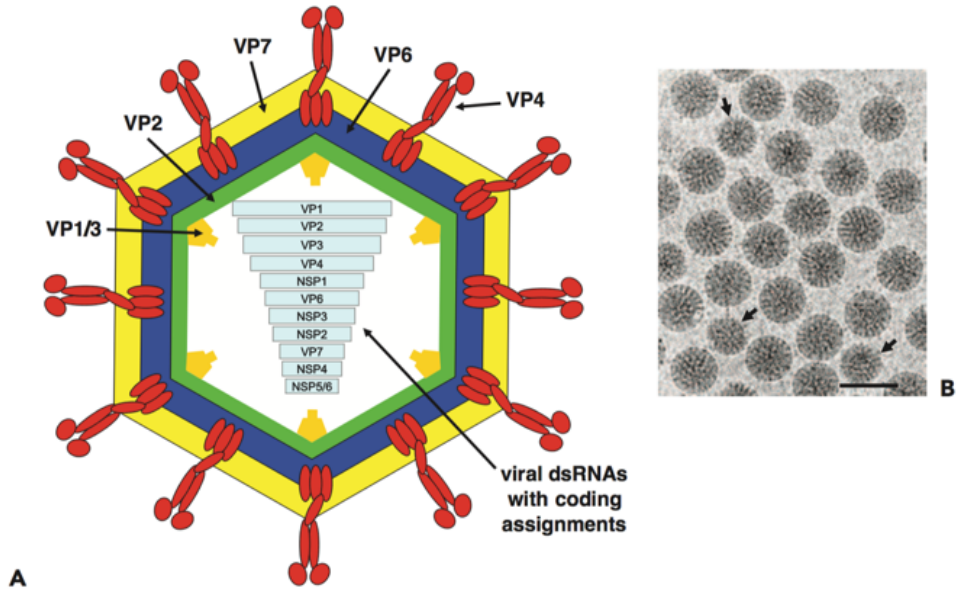

Figure 2.1. Schematic diagram and electron micrograph of Rotavirus particles.

Source: [11].

A: The particle is composed of three concentric protein shells (VP7, VP6, and VP2, shown in different colors) and the spike protein VP4 that spans the VP6 and VP7 layers and extends out from the particle. A transcription complex of VP1 and VP3 is inside the VP2 layer. The viral double-stranded RNA (dsRNA) genome is segmented.

B: Rotavirus triple-layered particles and a few double-layered particles (arrows) are easily visualized by electron microscopy. Bar, 100 nm.

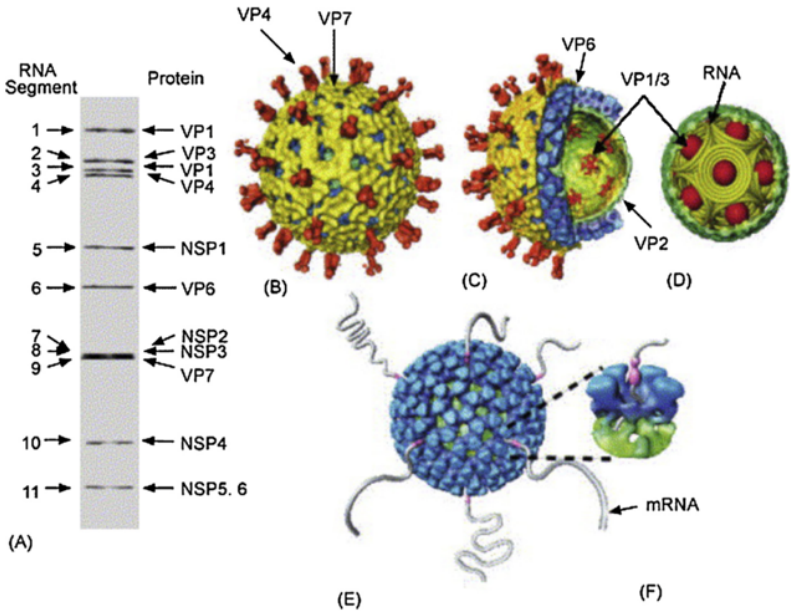

Figure 2.2. Rotavirus structures [15], [16].

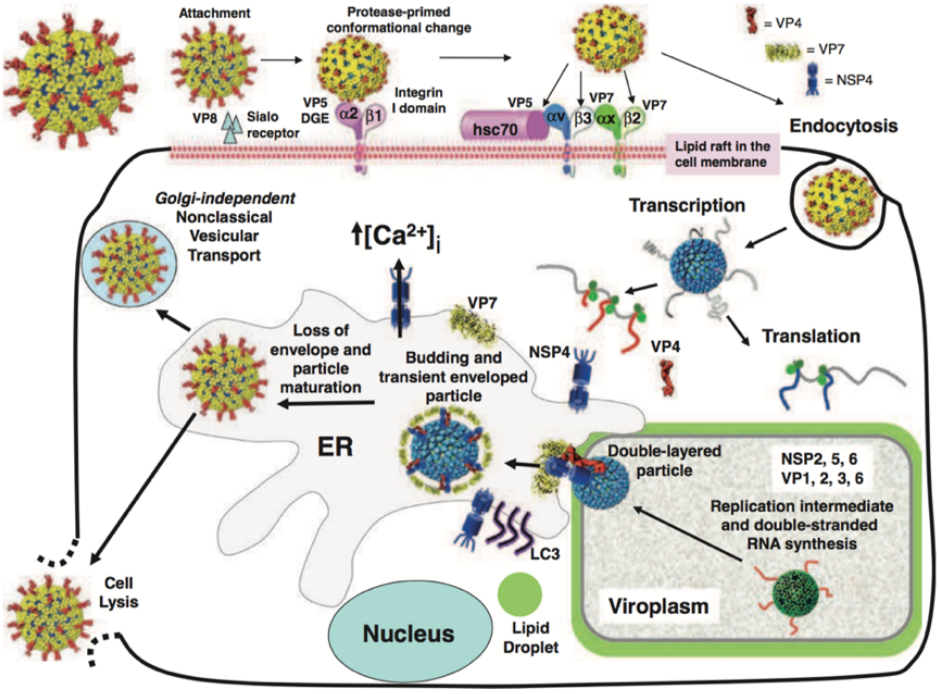

Figure 2.3. Schematic of the Rotavirus replication cycle [11]

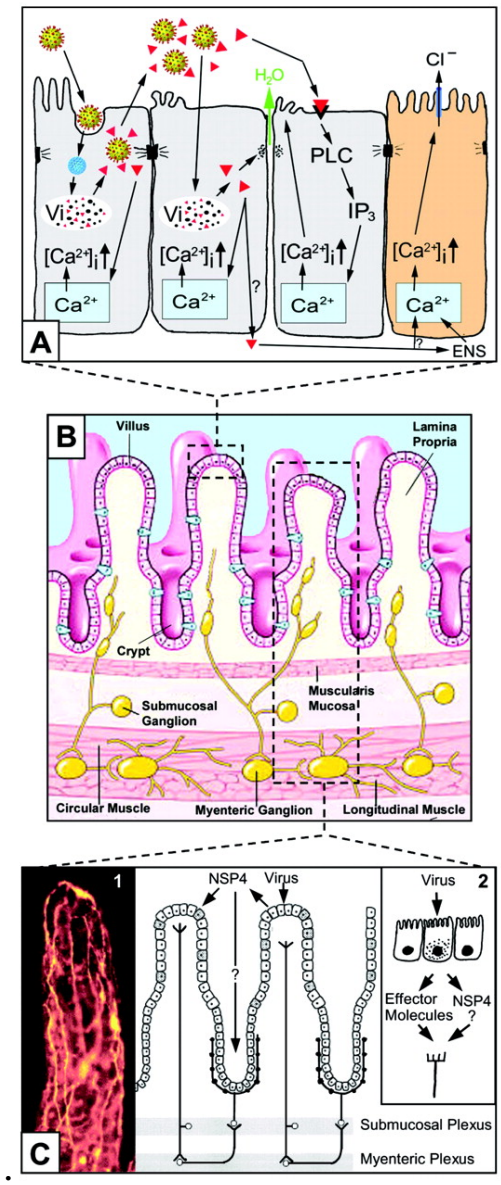

Figure 2.4. Model of Rotavirus-induced diarrhea.

Panel A depicts events in the infected epithelium (from left to right across the four cells).

Panel B shows the typical architecture of the small intestine, with the circulatory system removed for clarity. This panel emphasizes the ENS and its ganglia in the different submucosal levels [17], [18].

Panel C shows a reflex arc in the ENS that can receive signals from the villus epithelium and activate the crypt epithelium. Inset 1 shows a whole mount of an adult mouse small intestinal villus, stained with antibody to protein gene product 9.5 to reveal the rich innervation (yellow stain). Inset 2 shows that infected villus enterocytes may stimulate the ENS by the basolateral release of NSP4 or other effector molecules [18].

2.1.3. Replication

Figure 2.3 shows a schematic of the Rotavirus replication cycle. To summarize, the Rotavirus replication cycle includes the following steps:

- Attachment, mediated by VP4 and VP7；
- Penetration and uncoating；
- Plus strand ssRNA (=mRNA) synthesis, mediated by VP1, VP3 and VP2;
- Viroplasm formation, mediating RNA packaging, minus-strand RNA synthesis (=RNA replication) and DLP formation;
- Virus particle maturation (to TLPs) and release.

2.1.4. Classification

According to the serological reactivity and genetic variability of VP6, at least eight different groups, also termed species, are differentiated (termed RVA-RVH). The Rotavirus species comprises at least 36 G types (according to the nt sequence of VP7) and 51 P types (according to the nt sequence of VP4) [15], [19], [20].

- For G types, serotypes and genotypes are synonymous, for example, G1, G2.
- For P types, there are many more P genotypes than reference sera determining P serotypes: therefore, a double nomenclature has been introduced, for example, P1A[8] designating the P serotype 1A and P genotype 8 [11].

Table 2.1. Genotypes of species A Rotaviruses.

Rotavirus protein	Percent identity	Number of genotypes	Genotype
VP7	80	27G	Glycosylated
VP4	80	35P	Protease sensitive
VP6	85	16I	Inner capsid
VP1	83	9R	RdRp[c]
VP2	84	9C	Core protein
VP3	81	8M	Methyltransferase
NSP1	79	16A	Interferon Antagonist
NSP2	85	9N	NTPase

Rotavirus protein	Percent identity	Number of genotypes	Genotype
NSP3	85	12T	Translation enhancer
NSP4	85	14E	Enterotoxin
NSP5	91	11H	PHosphoprotein

Source: [11], [21].

2.1.5. Genotypes

Currently, 36 G and 51 P genotypes have been approved by the Rotavirus Classification Working Group [20]. A complete Rotavirus serotype is described using a number (or a number and letter) for the P serotype, followed by a number in brackets that represent the P genotype. It is followed or preceded by the G type designation (e.g., P1A[8]G1). Table 2 lists the most common human serotypes [12].

Table 2.2. Common Human Group A Rotavirus Serotypes Worldwide

VP4 Serotype (Genotypes)	Associated VP7 Types
P1A[8]	G1, G3, G4, G9, G12
P1P1B[4]	G2
P2[6]	G9, G12

Source: [12].

2.2. PATHOGENESIS

The mechanism by which Rotavirus induces diarrhea is poorly understood. The available reports describe various findings: malabsorption following the destruction of the epithelium [22], villus ischemia [23], the action of NSP4, a viral enterotoxin [24], and activation of the enteric nervous system [25]. Recently, the pathogenesis of the symptom vomiting has also been elucidated by observations that Rotavirus can infect the enterochromaffin cells in the gut and stimulate the production of 5- hydroxytryptamine (serotonin) which in turn activates vagal afferent nerves and stimulates brain stem structures controlling vomiting [15], [26].

Rotavirus infections are more likely to be severe in children three to 24 months of age than in younger infants or older children and adults. Several studies have offered possible explanations for these differences in age susceptibility. First, children of increasing age may be protected by a virus-specific immune response generated by repeated natural infections. Protection of young infants may be mediated by passively transferred, transplacental, maternal antibodies. Breastfeeding protects against Rotavirus disease. Second, infant mice have a larger percentage of intestinal epithelial cells with putative Rotavirus-binding proteins on the surface than do older mice - an observation that correlates directly with the age susceptibility to disease. Last, Rotavirus entry into target cells is facilitated by cleavage of VP4, which occurs in the presence of trypsin, elastase, or pancreatin. Quantities of these exopeptidases are decreased in intestinal fluid secretions of newborn infants compared with older infants and young children [12].

Children in developing countries are more susceptible to severe Rotavirus disease than those in developed countries. This is probably a consequence of poor access to hydration therapy and medical care, poor nutrition, concomitant infections, and possible differences in the gut microbiome [27]. Several studies in animals support the hypotheses that poor nutrition, or associated bacterial infections, may enhance the severity of Rotavirus-induced enteritis [12].

2.3. IMMUNITY AND PROTECTION

Protection against Rotavirus infection is mediated by both humoral and cellular components of the immune system. Following the first infection, the serological response is directed mainly against the specific viral serotype (for example, a homotypic response), whereas a broader, heterotypic antibody response is elicited following ≥1 subsequent Rotavirus infections [28].

A study that monitored 200 Mexican infants from birth to two years of age by weekly home visits and stool collections detected based on the fecal excretion of virus or a serologic response a total of 316 Rotavirus infections, of which 52% were first and 48% repeated infections. Children with 1, 2, or 3 previous infections had a progressively lower

risk of subsequent Rotavirus infection (adjusted relative risk, 0.62, 0.40, and 0.34, respectively) or diarrhea (adjusted relative risk, 0.23, 0.17, and 0.08) than children who had no previous infections. Subsequent infections were significantly less severe than first infections (p=0.02), and second infections were more likely to be caused by another G type (p=0.05) [29]. However, one study from India reported that the risk of severe disease continued after several reinfections [30].

Rotavirus is a ubiquitous infection in young children. In settings without Rotavirus vaccines, nearly all children are exposed to Rotavirus and acquire antibodies by 2 to 3 years of age, and most Rotavirus diarrhea occurs during the first three years. In the first three months, infections might be asymptomatic or less severe than those in older infants; this is most likely a result of the protective effect of passively transferred maternal antibodies or breastfeeding or both. Infections in neonatal nurseries are often asymptomatic. First infections after three months of age are generally associated with diarrhea that can range from mild to severe, whereas subsequent exposures lead to milder illness or asymptomatic infections [12].

2.4. CLINICAL MANIFESTATION, DIAGNOSIS

2.4.1. Clinical manifestation

In children, the incubation period for Rotavirus diarrhea is short, usually less than 48 hours. The clinical manifestations of infection vary and depend on whether it is the first infection or reinfection. The first infection after three months of age is generally the most severe. Infection may be asymptomatic, may cause self-limited watery diarrhea, or may result in severe dehydrating diarrhea with fever and vomiting. Up to one-third of infected children may have a temperature higher than 39°C. The gastrointestinal symptoms generally resolve in three to seven days. The clinical features and stool characteristics of Rotavirus diarrhea are nonspecific, and other pathogens may cause similar illnesses. As a result, confirmation of a diarrheal illness as Rotavirus requires laboratory testing [31].

In one study, a comparison was made between (a) the clinical manifestations of 78 patients hospitalized with Rotavirus diarrhea and (b) 72 patients hospitalized with a

diarrheal illness that was not associated with Rotavirus. The majority of both Rotavirus- and non–Rotavirus-infected children had a temperature of 37.9°C or above. Those with Rotavirus vomited and became dehydrated significantly more often than those who did not have Rotavirus. The mean duration of vomiting was longer in the Rotavirus-positive group than in those who did not have Rotavirus (2.6 days versus 0.9 days). Rotavirus diarrhea started later than vomiting but lasted longer (mean duration, five days versus 2.6 days). After infants and children were hospitalized, diarrhea continued for a mean of 2.6 days (range, 1 to 9 days) in the Rotavirus group and 3.8 days (range, 1 to 16 days) in the Rotavirus-negative group. The duration of hospitalization ranged from 2 to 14 days, with a mean of 4 days, for the patients infected with Rotavirus and for a mean of 6 days (range, 2 to 27 days) for the Rotavirus-negative group [11].

2.4.2. Complications

Rotavirus infection in infants and young children can lead to severe diarrhea, dehydration, electrolyte imbalance, and metabolic acidosis. Children who are immunocompromised because of congenital immunodeficiency or because of bone marrow or solid organ transplantation may experience severe or prolonged Rotavirus gastroenteritis and may have evidence of abnormalities in multiple organ systems, particularly the kidney and liver [31].

2.4.3. Diagnosis

Rotavirus can be detected in stool specimens from children with gastroenteritis by several techniques, including electron microscopy, polyacrylamide gel electrophoresis, antigen detection assays, reverse transcription-polymerase chain reaction (PCR), and virus isolation [32].

Children with gastroenteritis are not routinely tested for Rotavirus because the results do not alter treatment. When testing is performed, antigen detection tests - including commercially available enzyme-linked immunosorbent assays (ELISAs) and immunochromatographic assays - are widely used. Most of these tests have high sensitivity and specificity (90-95%) [33]. The window for the detection of viral shedding using ELISA

usually ends within one week after the onset of illness, but the virus can be detected for more extended periods by more-sensitive assays, such as PCR [34], [35].

PCR is widely used in research laboratories to detect the viral genome, which is more sensitive and permits the genotyping of virus isolates [34], [36]. It provides data on the VP7 and VP4 genotypes that form the basis of binary classification (G and P type, respectively) of Rotavirus strains [32].

2.5. TREATMENT

2.5.1. General information

A mild case of Rotavirus disease, where the child is active, shows no signs of dehydration, has had between zero and two vomiting episodes within 12 hours, has had a few loose or low output watery stools per day and has no fever or a low-grade fever, requires only observation. Symptoms can last for 1–5 days, but if they last for >1 week, medical consultation should be sought. Increasing and/or intense vomiting and repeated episodes of watery diarrhea (for example, >1 episode per hour, especially if abundant) are the main features that indicate the need for specific treatment.

WHO-recommended treatment, such as zinc supplements, oral rehydration therapy, and treatment with intravenous fluids, when needed, can help rehydrate children until the intestine can repair and recover [37], [38].

2.5.2. Fluid and electrolyte management

In low-income countries, particularly in hard-to-reach areas where children do not have timely access to such medical care, severe disease can result in rapid dehydration, leading to electrolyte imbalance, shock, and death. Nevertheless, many of the world's most impoverished children do not have access to oral rehydration therapy, despite it being inexpensive and effective. Oral rehydration therapy coverage hovers around 40% in many of the places where the highest number of diarrhea deaths occur [39]. The goal of treatment is avoiding or rapidly treating severe dehydration and maintaining protein-calorie intake to prevent death or worsening malnutrition. In middle-income and high-income countries, reducing hospitalization and the duration of diarrhea are the main goals.

2.5.3. Dietary management

Dietary management is an essential factor in the care of children with acute diarrhea [40]. In patients with dehydration, food withdrawal is advised for only 4–6 hours after initiating rehydration therapy [38], [41].

Breastfeeding should be encouraged and is never contraindicated. The administration of repetitive, small portions of regular undiluted milk formulas is recommended for infants and children >6 months of age.

The administration of lactose-free formulas might reduce the duration of treatment and the risk of treatment failure [40] and can be considered for selected children, such as those requiring hospitalization [38]. Importantly, the maintenance of adequate protein-calorie intake during the diarrhea episode using home-available, age-appropriate foods should be encouraged, especially in low-income settings [40].

2.5.4. Probiotics

Commonly used probiotics for the treatment of acute diarrhea are lactic acid-producing bacteria, such as Lactobacillus rhamnosus, Lactobacillus plantarum, several strains of Bifidobacteria and Enterococcus faecium (the SF68 strain), and yeast, such as Saccharomyces boulardii [42].

Most meta-analyses suggest a modest benefit of probiotics in reducing the duration of diarrhea by approximately one day and up to two days for Rotavirus-induced diarrhea, although studies have been performed mostly in middle-income and high-income countries, and some studies did not report a clear benefit [43], [44].

In low-income regions, treatment with probiotics has a positive immunomodulatory effect (that is, an increased anti-Rotavirus IgG response in individuals who received treatment compared with individuals who received a placebo), improves intestinal function in children with Rotavirus infection and might decrease repeat episodes of Rotavirus diarrhea [45], [46]. However, probiotics are not included in the standard of care for children with Rotavirus diarrhea globally.

2.5.5. Other drugs

Antiviral therapy for Rotavirus infection has been studied but remains mostly in preclinical stages. One exception is nitazoxanide, a broad-spectrum antiviral drug that has been reported to reduce the duration of diarrhea and the duration of hospitalization of children with acute Rotavirus diarrhea [47], [48], [49]. One study in hospitalized children five months to seven years of age reported a significant reduction in the median time to the resolution of all Rotavirus-associated gastrointestinal symptoms from 75 hours in children who received placebo treatment to 31 hours in children who received a 3-day course of nitazoxanide treatment [48].

Other potential therapies for Rotavirus gastroenteritis include racecadotril and smectite. However, treatment with racecadotril did not reduce the proportion of patients with diarrhea five days after treatment. Besides, one meta-analysis of seven clinical trials reported that racecadotril treatment is more effective than a placebo or no intervention at reducing the duration of illness and stool output in children with acute diarrhea [50].

Combination trials evaluating the simultaneous use of several treatments are lacking [51]. Indeed, improvements in treatment strategies are needed, especially in regions where Rotavirus-associated deaths occur and where vaccines are underutilized [35].

2.6. MODE OF TRANSMISSION [31]

2.6.1. Reservoir

The reservoir of Rotavirus is the gastrointestinal tract and stool of infected humans. Although Rotavirus infection occurs in many nonhuman mammals, the transmission of animal Rotaviruses to humans is believed to be rare and probably does not lead to clinical illness. Although immunodeficient persons may shed Rotavirus for a prolonged period, an actual carrier state has not been described.

2.6.2. Transmission

Person-to-person contact or exposure to aerosolized respiratory droplets are the most likely modes of transmission.

2.6.3. Communicability

Rotavirus is highly communicable, as evidenced by the nearly universal infection of children by age five years in the pre-vaccine era. Infected persons shed large quantities of virus in their stool beginning two days before the onset of diarrhea and for up to 10 days after onset of symptoms. Rotavirus may be detected in the stool of immunodeficient persons for more than 30 days after infection. Spread within families, institutions, hospitals, and child care settings are common.

2.7. ROTAVIRUS EPIDEMIOLOGY AND BURDEN OF DISEASE

2.7.1. Burden of disease

Rotavirus infects nearly every child by the age of 3–5 years and is the leading cause of severe, dehydrating diarrhea in children less than five years old worldwide. WHO estimated that in 2008, approximately 453,000 (420,000–494,000) Rotavirus gastroenteritis-associated child deaths occurred worldwide. These fatalities accounted for about 5% of all child deaths and a cause-specific mortality rate of 86 deaths per 100,000 population aged <5 years [1]. Rotavirus has a case-fatality rate (CFR) of approximately 2.5% among children in developing countries who present to health facilities. This CFR is higher in areas without proper access to health care [52].

In 2013, an estimated 215,000 deaths related to Rotavirus gastroenteritis occurred in children under five years old, in which 49% was accounted for four countries (Nigeria, Pakistan, India, and the Democratic Republic of the Congo) [2].

In a study reporting the results of the Global Rotavirus Surveillance Network (GRSN) from 2008 to 2016: 403,140 children younger than five years of age admitted to hospital with AGE from 349 sites in 82 countries, of whom 132,736 (32.9%) were Rotavirus-positive [53].

In more than 100 studies conducted around the world among children younger than five years of age who were hospitalized for diarrhea, Rotavirus was the most common cause of diarrhea (detected in 20–60% of cases). Prospective studies of children observed from birth to two years of age suggest that the incidence of Rotavirus diarrhea varies directly

with the intensity of surveillance. These studies indicated that 77% to 83% of children will have an episode of Rotavirus diarrhea in their first 2 to 3 years of life and, depending somewhat on the locale, seek medical attention or treatment [12].

In low-income countries the median age at the primary Rotavirus infection ranges from 6 to 9 months (80% occur among infants <1 year old) whereas, in high-income countries, the first episode may occasionally be delayed until the age of 2–5 years, though the majority still occur in infancy (65% occur among infants <1 year old) [1].

Infants and children with Rotavirus-associated gastroenteritis exhibited some dehydration, vomiting, and watery diarrhea, which indicate the severity of Rotavirus infection, more frequently than those with non-Rotavirus gastroenteritis [54]. There were significantly high rates of infection among children younger than two years old, especially those between 6 and 11 months old. The lower prevalence in older children could be due to acquired immunity through previous exposures [55].

2.7.2. Seasonality

In most low-income countries in Asia and Africa, Rotavirus epidemiology is characterized by one or more periods of relatively intense Rotavirus circulation against a background of year-round transmission, whereas in high-income countries with temperate climates a distinct winter seasonality is typically observed. This difference, as well as differences in health care availability and childhood co-morbidity, drive the marked inequality in Rotavirus disease burden between low- and high-income countries [1].

Most studies discovered that the distinct winter seasonality of Rotavirus hospitalizations in a temperate environment stands on the contrary to the year-round disease seen in tropical climates. It means that a child born in a temperate setting right after the Rotavirus season may have to wait many months before encountering the first possible natural infection in the following winter. On the contrary, a child born in a tropical climate may be exposed any day. Consequently, the average age at first infection is often younger in developing countries in tropical areas. In such tropical countries, the early episode of Rotavirus disease occurs most often during the first year of life, whereas frequently in

developed countries, the highest prevalence of first Rotavirus infections occurs in the second year of life. Therefore, an effective vaccine program in a developing country may require earlier and higher levels of coverage than in a developed country [12].

2.7.3. Genotype diversity

With the advent of Rotavirus genotyping by PCR, detailed studies of the Rotavirus molecular epidemiology have become possible. Genotyping has an enormous value for assessing the evolution and epidemiological pathways of Rotavirus in humans, mammals, and birds [15].

Currently, 36 G and 51 P genotypes have been approved by the Rotavirus Classification Working Group [20]. Despite the broad genomic and antigenic diversity of Rotavirus, globally, only a small number of Rotavirus types have prevailed in humans during the past three decades. Currently, 5 G-P combinations (G1P[8], G2P[4], G3P[8], G4P[8]) and G9P[8]) account for approximately 90% of all human Rotavirus infections in many parts of the world; type G1P[8] is the most prevalent combination. However, data from countries in Asia and Africa show greater strain diversity with several Rotavirus types circulating simultaneously [1], [15].

An example in Asia is in Indonesia, data from 2015-2018 indicated that the most common G-P combination was equine-like G3P[8] (70.8%), followed by equine-like G3P[6] (12.4%), human G1P[8] (8.8%), G3P[6] (1.5%), and G1P[6] (0.7%). The dynamic changes in Rotavirus genotypes from equine-like G3 to typical human G1/G3 in Indonesia can occur even in the country with low Rotavirus vaccine coverage rate [56].

For unexplained reasons, fecal specimens from children from developed countries are typically infected with a single strain of virus of one of the common serotypes found globally, while in developing countries, the rate of mixed infections with two or more strains can be as high as 30%. These mixed infections, as well as single-virus infections in developing countries, may include uncommon serotypes not found in other parts of the world [12].

The prevalent types may vary from one season to the next, even within the same geographical area; these natural variations are essential to keep in mind when monitoring ecologic changes in Rotavirus strains following the implementation of Rotavirus vaccination. The type of Rotavirus does not usually correlate with the severity of the disease. There are currently no known laboratory markers for Rotavirus virulence [1].

2.8. GLOBAL ROTAVIRUS SURVEILLANCE NETWORK

2.8.1. Objectives of the surveillance

For all countries, the primary objectives for the Rotavirus surveillance are to:

- Determine the epidemiology and burden of Rotavirus hospitalizations;
- Document the spectrum of clinical presentations and outcomes of Rotavirus cases;
- Determine the age and seasonal distribution of Rotavirus hospitalizations;
- Identify the prevalent, circulating strains of Rotavirus.

For countries that have yet to introduce the Rotavirus vaccine, an objective is to:

- Generate information to facilitate and support the introduction of the Rotavirus vaccine.

For countries that have introduced the Rotavirus vaccine, objectives are to:

- Monitor impact of Rotavirus vaccination on disease and changes in epidemiology and circulating strains after Rotavirus vaccine implementation
- Estimate vaccine effectiveness by using surveillance as a platform for special studies.
- An additional objective could include to monitor the burden of other enteric pathogens.

2.8.2. Case definitions and final classification [57]

2.8.2.1. Suspected case definition for case finding

Acute (<14 days) watery diarrhea, defined as three or more loose or watery stools in 24 hours in a child <5 years of age who is admitted for treatment of diarrhea to a hospital ward or emergency unit at a participating surveillance facility. Children with bloody diarrhea and nosocomial infections are excluded.

2.8.2.2. Confirmed case definition

A suspect case in whose stool the presence of Rotavirus is demonstrated by using an enzyme immunoassay (EIA) or PCR-based methods.

2.8.2.3. Special considerations

If diarrhea surveillance is also intended to identify other enteric pathogens, then some components of the suspect and confirmed case definition might change. For example, bloody diarrhea might be included.

2.8.3. Specimen collection

The whole stool is the preferred specimen. Collect a minimum of 1 mL of stool for basic confirmatory testing; 2 mL or more may be needed for additional testing, such as genotyping. A stool specimen should be obtained within 48 hours of hospital admission to avoid the detection of nosocomially acquired infections. Avoid using rectal swabs or swabs placed in bacterial media, which are not optimal for Rotavirus detection or characterization.

Stool specimens should be placed in sterile screw-top containers, properly labeled. Samples can be stored temporarily at 4–8°C for up to one month. Ice packs can be used to keep samples cool. Freeze-thaw cycles should be avoided where possible. If prolonged storage is necessary, store at -70°C, as evidence suggests that the ability to characterize Rotaviruses declines during storage for years at -20°C.

If stool samples are also tested for bacterial or parasitic pathogens by conventional methods, the specimens should be transported to the lab within two hours of collection and placed on appropriate media. Specimens should then be stored in a freezer at -20°C or colder until testing is performed.

2.8.4. Laboratory testing

2.8.4.1. Confirmation methods

EIAs are most commonly used for Rotavirus detection in the stool. Several EIA kits (PremierTM Rotaclone®, ProSpecTTM, and RIDASCREEN®) are available. Follow the manufacturer's procedures for each kit. The sensitivity of EIAs is 75–82%, with 100% specificity [58]. Thus, occasional false negatives are possible, particularly at lower viral loads, though the clinical significance of Rotavirus at concentrations below the threshold of EIA detection is unclear [59].

2.8.4.2. Additional testing

Confirmatory testing of EIA results may be done by testing for the presence of the VP6 gene using PCR or NP6 and NSP3 genes by real-time reverse transcription PCR (RT-PCR). Rotavirus strain characterization is done by using RT-PCR to identify both G and P types. A subset of Rotavirus-positive stools obtained from routine surveillance should be chosen for strain characterization. It is recommended that a minimum of 50–60 randomly selected specimens per year be genotyped from each country. The randomly selected sample should be proportional to the age and seasonal distribution of cases. Only specimens > 3mL should be chosen to avoid running out of material. All non-typeable isolates should be sent to an appropriate reference laboratory for sequencing.

2.8.4.3. Laboratory quality control and assessment

A standard proficiency panel of Rotavirus-positive and -negative stool samples can be obtained from the global or regional Rotavirus laboratories. Labs should also arrange to send some Rotavirus-positive stool specimens to a regional laboratory for independent confirmation of results. External Quality Assessment and Quality Control of the laboratory should be completed annually.

2.8.4.4. Laboratory networks

Participation in a Rotavirus surveillance network is voluntary. The Global Rotavirus Laboratory Network has more than 100 participating laboratories throughout the world [60]. The network focuses on conducting high-quality diagnostic testing for

Rotavirus diarrhea and characterizing the most prevalent strain genotypes in different countries and regions. The network promotes standardization of data collection and laboratory quality and control through a global external quality assessment program coordinated by WHO. As of December 2017, the network includes 187 laboratories, including 108 sentinel hospital laboratories, 69 national and provincial laboratories, 9 Regional Reference Laboratories, and one Global Reference Laboratory.

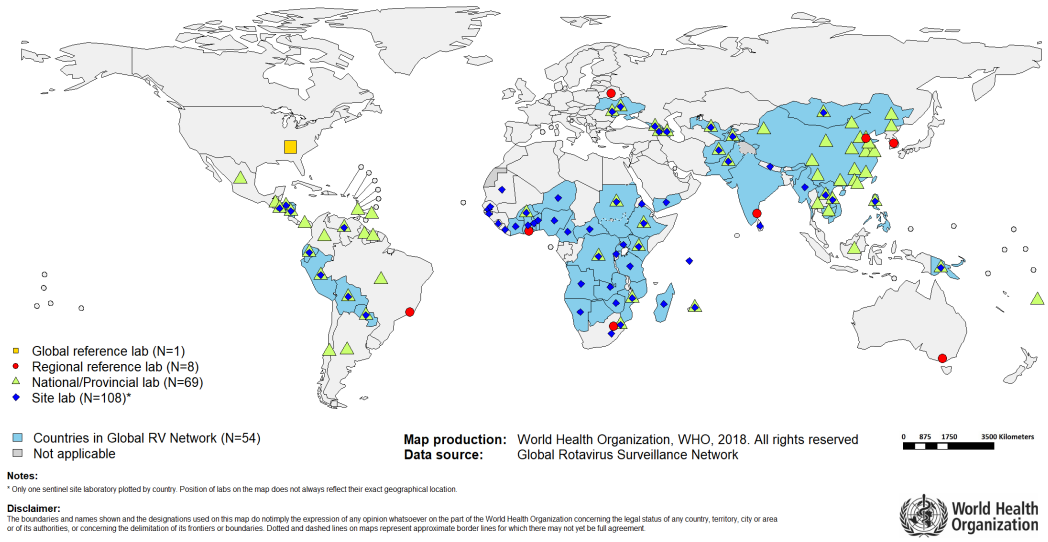

Figure 2.5: WHO Member States that reported data to the Global Rotavirus Surveillance Network, 2018.

2.9. ROTAVIRUS VACCINE

2.9.1. General information

WHO recommends Rotavirus vaccines should be included in all national immunization programs and considered a priority, particularly in countries with high Rotavirus gastroenteritis-associated fatality rates, such as in south and south-eastern Asia and sub-Saharan Africa. Rotavirus vaccine was introduced in 101 countries by 2018, including four in some parts of the country. Global coverage was estimated at 35% [7].

Four Rotavirus vaccines are currently available on the global market, but most are very expensive - around US$45 per dose in the private market. This cost is prohibitive for most low- and middle-income countries. A key strategy for creating and maintaining a long-term, financially sustainable supply of Rotavirus vaccines is for additional manufacturers - especially those based in emerging countries - to develop Rotavirus vaccines and enter the marketplace.

In Vietnam, locally state-owned vaccine manufacturer POLYVAC produced a licensed Rotavirus vaccine - Rotavin-M1 - that is available in the domestic private market, and more than one million doses have been administered to infants across Vietnam since 2012. The price of this Vietnamese-made vaccine is one-third the cost of an imported vaccine, at just $20 per dose [8] (Table 2.3).

2.9.2. Vaccine efficacy

Published randomized clinical trials are not adequately powered to conclude whether or not efficacy wanes for either Rotarix or Rotateq.

With Rotateq, one randomized clinical trial that enrolled subjects from 11 countries, reported efficacy against severe disease estimated at 98% (95% CI: 88%–100%) during the first Rotavirus season and 88% (95% CI: 49%–99%) during the second season. An extension of this trial demonstrated a sustained reduction in the number of hospitalizations for Rotavirus disease also three years after vaccination [1].

Reports from randomized clinical trials were consistent with little decrease in the efficacy of Rotarix against severe Rotavirus disease during the second season of follow-up, from 83% (95% CI: 67%–92%) to 79% (95% CI: 66%–87%) in Latin America and from 96% (95% CI: 90%–99%) to 86% (95% CI: 76%–92%) in Europe. A randomized clinical trial of Rotarix conducted in three high-income settings in Asia reported sustained efficacy against severe Rotavirus gastroenteritis of 100% (95% CI: 67.5%–100%) during the third year of life [1].

Table 2.3. Three vaccines licensed for use in Vietnam.

Vaccine name	Rotarix	Rotateq	Rotavin-M1
Manufacturer	GlaxoSmithKline Belgium	Merck & Co., Inc USA	POLYVAC Vietnam
License	Licensed 2004; WHO prequalification Jan 2007 (vial) Mar 2009 (tube and applicator)	Licensed 2006; WHO prequalification Oct 2008	Nationally licensed 2007
Formulation	Monovalent attenuated human Rotavirus strain	Pentavalent, human-bovine reassortant vaccine	Monovalent attenuated human Rotavirus strain
Strains present in the vaccine	G1P[8]	G1, G2, G3, G4, and P[8]	G1P[8]
Protect against other strains?	Yes, broad protection demonstrated	Yes, broad protection demonstrated	N/A
Efficacy against severe Rotavirus diarrhea in children <1 year (high-income countries)	95.8–100%	85–96%	N/A
Efficacy against severe Rotavirus diarrhea in children <1 year (low- and midle-income countries)	49–85%	51–64%	IgA: 80.6% IgG: 72.8-85.3%
Dosage	At least 106 of live attenuated human G1P[8] particles per dose	A minimum titer of approximately 2.0 to 2.8 x 106 infectious units per reassortant	At least 2x106 of live attenuated human G1P[8] particles per dose

Vaccine name	Rotarix	Rotateq	Rotavin-M1
		and not greater than 116 x 106 infectious units per aggregate dose	
Schedule	2-dose Given on the same schedule as DPT1 and 2 vaccine doses	3-dose Given on the same schedule as DPT1, 2 and 3 vaccine doses	2-dose Minimum at 6 weeks, At least 30 days apart
Presentation	1. Liquid vaccine in oral, single-dose applicator 2. Liquid vaccine in squeezable, polyethylene single-dose tube 3. Lyophilized vaccine, reconstituted with CaCO3 buffer, oral applicator	Liquid vaccine in oral, squeezable tube	1 dose liquid vaccine in glass vial
Shelf life	36 months	24 months	24 months at -25 to -15°C 2 months from thawing at 2-8°C
Vaccine vial monitor on label	Yes	No	No
Storage requirements	2–8°C, not frozen and protected from light	2–8°C, not frozen and protected from light	-25 to -15°C
Safety: clinical studies (intussusception risk)	No increased risk detected	No increased risk detected	No increased risk detected

Vaccine name	Rotarix	Rotateq	Rotavin-M1
Safety: post-introduction (intussusceptio n risk)	Low-level risk in some countries, not in others	Low-level risk in some countries, not in others	N/A
Price in Vietnam	38.5$	29$	20$
Packaging			

Sources: [31], [61], [62].

2.9.3. Vaccine impact

Globally, Rotavirus prevalence among children younger than five years of age admitted with AGE to hospitals or emergency units decreased by nearly 40% in countries after the introduction of Rotavirus vaccines into their national immunization programs. In contrast, no such reduction was observed in regions where it was not introduced. Reductions by WHO regions ranged from 26.4% (15.0–37.8) in the Eastern Mediterranean Region to 55.2% (43.0–67.4) in the European Region and were sustained in nine countries (contributing up to 31 sites) for 6–10 years. [53]**.**

Many studies identified the age distribution of children with Rotavirus gastroenteritis shifted towards older children after the Rotavirus vaccine introduction. The study based on Global Rotavirus Surveillance Network from 2008-2016 indicated that in the pre-vaccine period, 17.8% of Rotavirus gastroenteritis cases occurred in the 0–5-month age group, 38.8% in the 6–11-month age group, 29.7% in the 12–23-month age group, and 13.7% in the 24–59-month age group. In the post-vaccine period, the proportion of Rotavirus gastroenteritis cases occurring in the 0–5-month age group decreased to 12.9% and that for 6–11-month age group decreased to 31.9%, whereas the proportion increased

for both the 12–23-month age group (to 36.4%) and the 24–59-month age group (to 18.8%; $p<0.0001$). An example of this trend is in Rwanda, the cumulative age distribution of Rotavirus gastroenteritis cases showed a rightward shift, with 56% of Rotavirus gastroenteritis hospital admissions occurring among infants in the pre-vaccine period compared with 31% after Rotavirus vaccine introduction [63]. Similarly, in Bolivia, there was a decrease in the proportion of Rotavirus gastroenteritis cases occurring by 12 months of age, from 67% in the pre-vaccine period to 55% in the post-vaccine period [64]. All remain to be seen whether this shift in the proportion of Rotavirus gastroenteritis cases to older ages is a reflection of improved protection shortly after vaccination, whether this shift will diminish over time as all cohorts up to fivwfive years of age are vaccinated, or whether the absolute number of Rotavirus gastroenteritis cases will change. Additionally, there might be differential enrollment practices by age between pre-vaccine and post-vaccine countries that would affect this age distribution, which would need further study [53].

Introduction of Rotavirus vaccination may be closely associated with the strain selection of Rotavirus genotypes [65], [66]. Besides, in Australia, the equine-like Rotavirus strain was found to be more common in areas where the Rotarix vaccine was being administered. These findings raise the possibility that the Rotarix vaccine is associated with the emergence of the equine-like strains and that Rotavirus vaccines may induce selective pressure that favors specific genotypes [65]. The G2P[4] genotype that re-emerged after the Rotavirus vaccine introduction in certain areas [67]. In rare instances, reassortment between RotaTeq vaccine component strains of genotypes P7[5]G1 and P1A[8]G6 have been observed to occur during human in vivo replication. This vaccine-derived reassortant has been demonstrated to be transmissible and is capable of causing symptomatic gastroenteritis. Typically in the post-licensure vaccine era, this reassortant has been detected among 1–3% of detected Rotavirus surveillance cases that have undergone sequencing [31].

2.10. VIETNAM SITUATION

2.10.1. Epidemiology

Diarrheal diseases remain one of the major killers of children under five years old in Viet Nam [68], with 7.0 percent of under-5 children reportedly having diarrhea two weeks prior to Vietnam Multiple Indicator Cluster Survey 2014 [69, 70]. Management of diarrheal diseases among children remained relatively ineffective; only 57.8% of children with diarrhea were treated for dehydration [69].

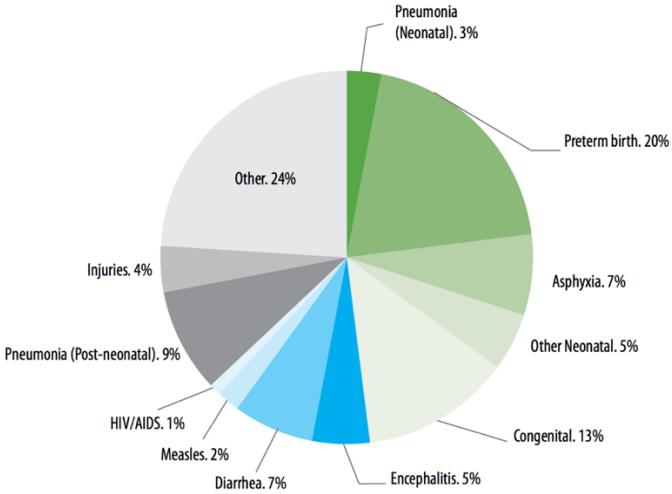

Figure 2.6. Causes of child death, 2012 [71] .

Estimates from 2013 determined that Vietnam had a total of 2,083 diarrhea-related deaths, of which 49.9% were due to Rotavirus. Rotavirus accounted for 3.1% total death of children under five years old [2]. Vietnam also had one of the highest Rotavirus hospitalization figures in South East Asia [3].

In 1998, Vietnam initiated Rotavirus surveillance under the WHO's auspices. The national surveillance conducted in 4 hospitals located across the country. In the nearest study from this sentinel surveillance, Rotavirus was detected in 46.7% of 8,889 children under five years of age enrolled in the surveillance. Rotavirus was detected year-round, but most Rotavirus gastroenteritis cases (77.1%) occurred between December and May,

corresponding to the Rotavirus seasonality. It is also found that the peaks varied by region. Rotavirus positivities varied between the youngest and oldest age, but children 6–11 months old (38.8%) and 12–23 months old (38.4%) counted for most cases. There were a significant higher times of diarrhea within 24 hours (8.3 times, 95%CI: 8.1–8.4 times) and higher proportion of severe dehydration (12.9%) in Rotavirus positive group than that in negative group (7.7 times, 95% CI: 7.6–7.9 times; and 9.7%, respectively). A downtrend of prevalence of G1P[8] was observed from 82% in 2013 to 15% in 2015. G2P[4] was found in 5% of samples in 2012, 9% in 2013, 36% in 2014, and 28% in 2015 [4].

Meanwhile, another study conducted the same period in five provincial hospitals (2012-2015) revealed the Rotavirus prevalence at 50.2% (678 Rota-positive/1,350 acute diarrhea cases). The common human G1P[8] (32.2%) and G2P[4] (13.0%) strains were most predominant, less predominant was G8P[8] strain (10.5%). The G8P[8] lineage was not detected in samples until 2014 than 2015 witnessed a rocket of this genotype from 5.2% in 2014 to 44.8% in 2015. Full genome sequencing and phylogenetic analysis of G8P[8] sequences reveal that this lineage represents a non-reassortant, monophyletic clade closely related to other G8P[8] strains isolated recently in Europe and Asia, and has experienced an unprecedented spread across Vietnam within a short period [72].

An economic evaluation of Rotavirus disease burden conducted in Vietnam in 2003 by Fischer et al. estimated that the Rotavirus disease burden is equivalent to an economic burden of an estimated $3.1 million in direct medical costs, $685,000 in direct nonmedical costs, and $1.5 million in indirect costs [5]. In 2009, data were collected from hospitalized children under five years old with symptoms of AGE was estimated that the costs of patients with and without Rotavirus were $217 and $158, respectively [6].

2.10.2. Rotavirus vaccination in Vietnam

The study of Fischer et al. in 2003 found that universal Rotavirus vaccination in Viet Nam can prevent 70 percent of the outpatient visits, 84 percent of hospitalizations, and 83 percent of the related deaths [5]. Universal Rotavirus vaccination in Viet Nam can significantly reduce the burden of Rotavirus infections and diarrhea in children [71].

However, to date, there is no available data about Rotavirus vaccine coverage in Vietnam. The country has been licensed two WHO-prequalified vaccines and one national-licensed vaccine:

Rotarix (GlaxoSmithKline, Rixensart, Belgium), which is based on the human Rotavirus G1P[8] strain, and RotaTeq (Merck and Company, Whitehouse Station, New Jersey, USA), consisting of 5 human-bovine recombinant strains.

The Vietnamese local-state Rotavin-M1 vaccine, which is based on the G1P[8] strain, is similar to the monovalent Rotarix vaccine, but the viral strain of the Rotavin-M1 vaccine is different from the Rotarix strain in both viral sequence and replication ability compared to the viral strain used in Rotarix. Rotavin-M1 has been available on the Vietnamese private market since 2012.

However, Rotavin-M1 requires frozen storage at -20°C. It presents significant challenges for the vaccine cold chain in Vietnam, which was designed to keep vaccines at 2-8°C. A second-generation formulation of their Rotavirus vaccine (called Rotavin) is evaluated, which does not need to be kept frozen, in a clinical trial in Vietnam. Rotavin contains the same Rotavirus strain as Rotavin-M1 and is produced in the same way, but only needs to be stored at 2-8°C. It is more compatible with the cold chain currently in place in Vietnam, allowing for more accessible storage and transport. It will also make the vaccine more attractive for use in many low- to middle- income settings, including in Vietnam. If the trial successfully confirms that Rotavin is as safe and immunogenic as the currently licensed frozen formulation, POLYVAC will apply for licensure of the new vaccine in Vietnam, and the government of Vietnam will consider its inclusion in the national immunization program. Besides, it may be possible in the future for POLYVAC to export Rotavin for use in other countries in the Mekong region.

Improving access to Rotavirus vaccines in Vietnam will not only save children's lives but also pave the way for a more sustainable and affordable Rotavirus vaccination program in the country to lessen the massive economic and health burden of Rotavirus disease.

CHAPTER 3:

MATERIALS AND METHODOLOGY

3.1. STUDY DESIGN

This study was a retrospective, hospital-based study conducted from 2013 to 2018 at Children Hospital No.1 in Ho Chi Minh city, a site of the Vietnamese Rotavirus Sentinel Surveillance, in collaboration with the Pasteur Institute in Ho Chi Minh City.

3.2. STUDY AREA

3.2.1. Southern Vietnam

Vietnam is a country in Southeast Asia, which has 93,67 million population, and the total fertility rate is 2.04 children born per woman (2017). In the north, the climate is monsoonal with four distinct seasons (Spring, Summer, Autumn, and Winter) while in the south (areas south of the Hải Vân Pass), the climate is tropical monsoon with two seasons (rainy and dry) [73].

The Vietnamese disease surveillance system divided into four administrative regions: Northern (28 provinces), Central (11 provinces), Highland (4 provinces), and Southern Vietnam (20 provinces) [74].

Table 3.1. Southern Vietnam general informations

Administrative region	Provinces	Area (km^2)	Population (2019)
Southeast (Đông Nam Bộ, Miền Đông)	Ba Ria-Vung Tau, Binh Duong, Binh Phuoc, Dong Nai, Ho Chi Minh city, Tay Ninh, Lam Dong	33,355.5	20,653,028
Southwest (Tây Nam Bộ, Miền Tây) or **Mekong River Delta** (Đồng Bằng Sông Cửu Long)	An Giang, Ben Tre, Bac Lieu, Ca Mau, Can Tho, Dong Thap, Hau Giang, Kien Giang, Long An, Soc Trang, Tien Giang, Tra Vinh, Vinh Long.	40,576.00	21,015,795

Source: [75]

Figure 3.1. Pasteur Institute in Ho Chi Minh city.

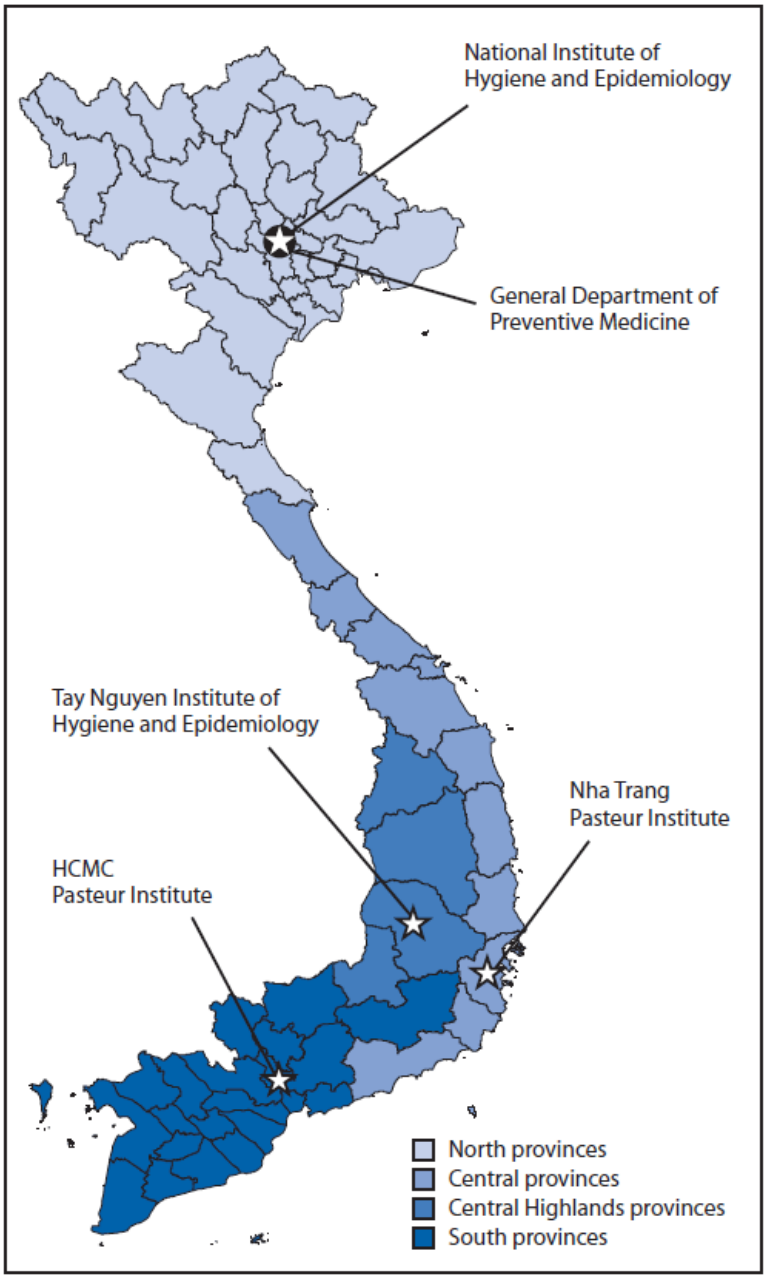

Figure 3.2. The four regional public health institutes and their provinces of responsibility [74].

3.2.2. Children's Hospital No. 1 in Ho Chi Minh City

Children's Hospital No. 1 in Ho Chi Minh City is a major pediatric hospital serving children in Ho Chi Minh City and southern Vietnam. The 1,400-bed hospital provides the most specialized neonatal care in the South, assigned by the Ministry of Health to direct the route to the South West region and to implement the Satellite Hospital Project. The hospital is also a collaborative clinical pediatric clinic with leading hospitals and institutes throughout the country and the World Health Organization.

The criteria to select this hospital were:

- To represent of geographical character for the South of Vietnam;
- To have the largest catchment population in the region;
- To have a sufficient number of inpatients;
- To have the capacity of human resources and logistics at hospitals.

BỆNH VIỆN
NHI ĐỒNG 1

Figure 3.3. Children's Hospital No. 1 in Ho Chi Minh City.

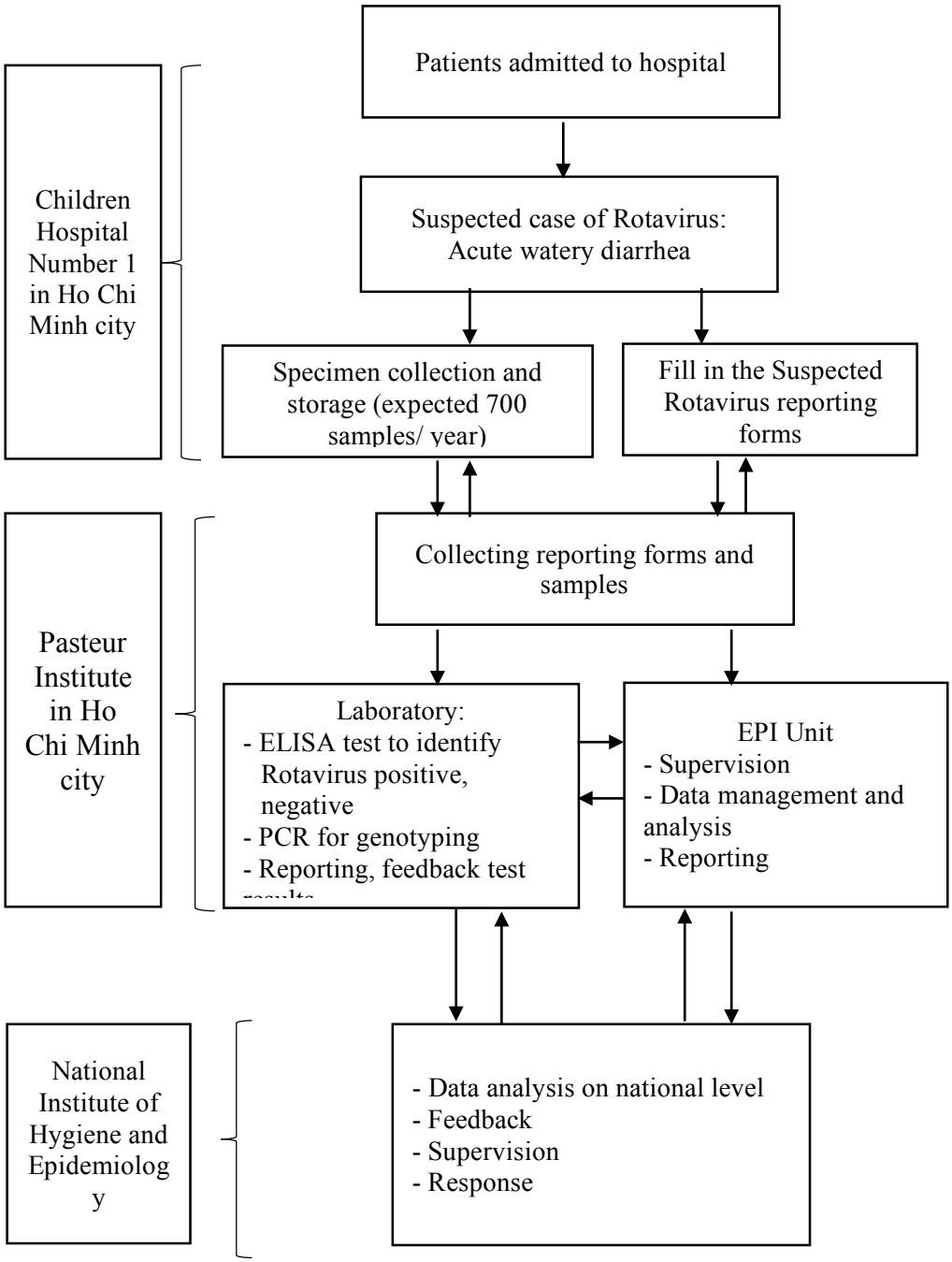

Figure 3.4. Flow diagram of Rotavirus sentinel surveillance in Southern Vietnam.

3.3. SAMPLING

3.3.1. Sampling method

In Children Hospital No.1, the number of hospitalized acute diarrhea is high. So we could not include all the cases into the sentinel surveillance. The selection rule was:

- For each case enrolled in the surveillance, hospital staff filled in the reporting form (Appendix 1) and collected the stool samples.
- Expected enrolled cases for 15-20 cases per week, about 70 cases per month.
- Selection method: Hospital staffs collected stool samples and reporting forms from the beginning of the week until the expected cases were reached.

3.3.2. Included criteria

For the sentinel surveillance, the definition of eligible children was children between 0 and 59 months of age whose primary reason for hospitalization was acute watery diarrhea, defined as ≥3 loose stools within 24 hours for <14 days between January 2013 and December 2018.

3.3.3. Exclusion criteria

- Bloody diarrhea;
- Diarrhea lasting ≥14 days before hospitalization;
- Acquired acute watery diarrhea during hospitalization;
- Parents/guardian does not allow their child to enroll in the surveillance.

3.4. DATA COLLECTION

Using a standard case definition and case-based data collection tool, hospital staffs prospectively identified children younger than five years of age admitted to the hospital or emergency unit with AGE to enroll in the surveillance. The informed consent was done by health workers, and the children were enrolled only when their parents or guardians agreed to participate in the surveillance.

Enrolled children underwent a routine medical examination by a pediatrician, including the assessment of dehydration status and temperature.

Demographic data, medical history, and clinical presentation/characteristics, vaccination history, were collected by the interview with the subjects' parents/guardians.

Reviews of medical records collected information such as date of discharge, disease outcomes, and discharge diagnosis. All information was written into the standardized case investigation form. After consent, a stool specimen was obtained from each child enrolled within 48 hours after admission for laboratory confirmation of Rotavirus.

3.5. SPECIMEN STORAGE AND LABORATORY TESTING

Fecal specimens were collected and stored at 20^{o}C after collection by Children Hospital No.1, then were shipped to Pasteur Institute in Ho Chi Minh city, which located approximately three kilometers from the Hospital.

At Pasteur Institute in Ho Chi Minh city's Laboratory, specimens were tested for Rotavirus by a commercial ELISA ProSpecT (Oxoid; Basingstoke, UK). At least twenty-five percent of ELISA positive samples randomly underwent P and G genotyping with a semi-nested multiplex reverse-transcriptase polymerase chain reaction using methods described in the WHO manual [57].

3.6. STUDY VARIABLES

3.6.1. Demographics characteristics

- Age (in months): Divided into four groups (<6 months, 6-11 months, 12-23 months, 24-59 months).
- Gender (male/ female).
- Time of admission: month, year.
- Geographic information:
  - Province;
  - Geographic regions: South-eastern region (7 provinces: Ba Ria-Vung Tau, Binh Duong, Binh Phuoc, Dong Nai, Ho Chi Minh city, Tay Ninh, Lam Dong), South-western (13 provinces: An Giang, Ben Tre, Bac Lieu, Ca Mau,

Can Tho, Dong Thap, Hau Giang, Kien Giang, Long An, Soc Trang, Tien Giang, Tra Vinh, Vinh Long), and other regions (for children had address in other provinces).

3.6.2. Clinical data

- Presence of vomiting. If present:
  + Maximum number of vomiting episodes in 24 hours at the peak of illness;
  + Duration of vomiting in days.
- Dehydration (none, mild-moderate, severe).
- History of fever (Yes/No). Maximum body temperature.
- A maximum number of diarrhea episodes in a 24-hour period, at the peak of illness.
- Duration of diarrhea (days).
- Treatment: oral rehydration solution (ORS), intravenous fluids (IV), antibiotics, probiotics, others (specify).
- The case outcome at discharge (discharged alive, alive with sequelae, died, transferred, left/ discharged against medical advice, unknown).
- Duration of hospitalization.
- Mortality.

3.6.3. Vaccination history

- Rotavirus vaccination history before infection (yes/no).
- Source of vaccination information (vaccination card, medical records, clinic logbook, maternal recall, other).
- If yes:
  + Type of Rotavirus vaccine (Rotarix, RotaTeq, Rotavin-M1, unknown)；
  + Number of doses received;
  + Dates received;
  + Status of immunization (Completed, Incompleted, Unknown).

3.6.4. Specimen

- Was the stool specimen collected from the case?
- Stool specimen ID (to be provided if stool specimen ID differs from unique case ID).
- Date of stool collection from the case.
- ELISA test was done on stool specimen:
  + Date of ELISA test on stool specimen;
  + ELISA results for stool specimen (positive, negative, indeterminate).
- PCR Genotyping done (on a subset of specimens):
  + Date when genotyping was performed;
  + Genotyping results (G-type);
  + Genotyping results (P-type).

3.7. ETHICAL CONSIDERATIONS

The sentinel surveillance was approved by the Ministry of Health for the currently applicable regulatory requirements. Because this was a retrospective review of sentinel surveillance, new written informed consent from caregivers was not obtained. Patient names were not used; instead, unique identification codes were used in order to ensure confidentiality.

3.8. DATA ANALYSIS

Data gathered from sentinel hospitals were entered into an online software of Microsoft Office Access 2010 for six years from 2013 to 2018. We used Microsoft Office Excel 2010, SPSS 20.0 for our analyses. The map of Southern Vietnam was designed by ARCGIC.

Children who did not meet with inclusion criteria or specimen failure will be excluded from the analysis.

Regarding geographic regions, based on the divided of the Vietnamese disease surveillance system, we divided subjects into three groups: South-eastern region (7

provinces: Ba Ria-Vung Tau, Binh Duong, Binh Phuoc, Dong Nai, Ho Chi Minh city, Tay Ninh, Lam Dong), South-western (13 provinces: An Giang, Ben Tre, Bac Lieu, Ca Mau, Can Tho, Dong Thap, Hau Giang, Kien Giang, Long An, Soc Trang, Tien Giang, Tra Vinh, Vinh Long), and other regions (for children had address in other provinces).

Regarding month of admission, to emphasize the seasonal trend, we divided into two groups: Dry season (November to April), and Rainy season (May to October).

Categorical variables were compared using Chi-square tests, and continuous variables with normal distributions were compared using the ANOVA test. Records with missing values were excluded while running the statistical analysis for this variable. Mean and 95% confidence interval of continuous variables were estimated from the observed.

Vesikari scale

We assessed the severity of diarrheal symptoms by using the Vesikari Clinical Severity Scoring System. It is currently recognized as the most accurate system for use in developing country vaccine trials [76]. It is a composite measure that relies on the clinical presentation profile of Rotavirus to identify severe Rotavirus gastroenteritis episodes, in which a higher score indicates increased disease severity.

There are seven scoring parameters included in the Vesikari Clinical Severity Scoring System. These parameters take into account each of the symptoms identified as important in the clinical presentation profile: diarrhea, vomiting, fever, dehydration, and the duration of diarrhea and vomiting. An additional parameter considered is treatment status. The seven parameters and the corresponding scores provided for each categorical level of severity are outlined in Table 3.2. Severity scores above 10 points (i.e., ≥11 points) are considered severe, scores between 7 and 10 are moderate, and scores less than 7 are mild (Table 3.3) [76].

Table 3.2. Vesikari clinical severity scoring system parameters and scores.

Parameter	**1**	**2**	**3**
Diarrhea			
Maximum number stools per day	1-3 times	4-5 times	≥ 6 times
Diarrhea duration (days)	1-4 days	5 days	≥ 6 days
Vomitting			
Maximum number per day	1	2-4 times	≥ 5 times
Vomitting duration (days)	1	2	≥ 3 days
Maximum body temperature	37.1-38.4	38.5-38.9	≥ 39
Dehydration	N/A	1-5%	**≥ 6%**
Treatment	ORS	Hospitalization	N/A

Table 3.3. Vesikari clinical severity scoring system severity rating scale.

Level	**Mild**	**Moderate**	**Severe**
Score	<7	7–10	≥11

Vaccine effectiveness:

Children were included in the VE analysis if they were aged at least six months at the time of notification. This age cut off for the analysis was applied to avoid the inclusion of young infants notified as having Rotavirus disease but who had vaccine virus shedding. A dose was considered valid if the Rotavirus infection notification date was >14 days after receipt of the Rotavirus vaccine to allow for time to develop a protective immune response.

We compared the VE against Rotavirus AGE of the completed vaccination group with the unvaccinated group. For genotype subgroup, we derived VE using the defined subgroup as cases and comparing Rotavirus-negative controls.

The VE against Rotavirus AGE was calculated using the formula:

$$VE = (1 - \text{crude OR}) \times 100\ (\%).$$

Conditional logistic regression models were used to determine the OR for the VE calculations.

CHAPTER 4:

RESULTS

4.1. CHARACTERISTIC OF THE STUDY POPULATION

Of 5,254 children with acute diarrhea extracted from the sentinel surveillance, 5,179 cases were included in the study. Seventy-five cases were subsequently excluded because of some reason describing in the flow diagram. 2,424 ELISA positive Rotavirus stools were identified, accounted for 46.80% of the study sample.

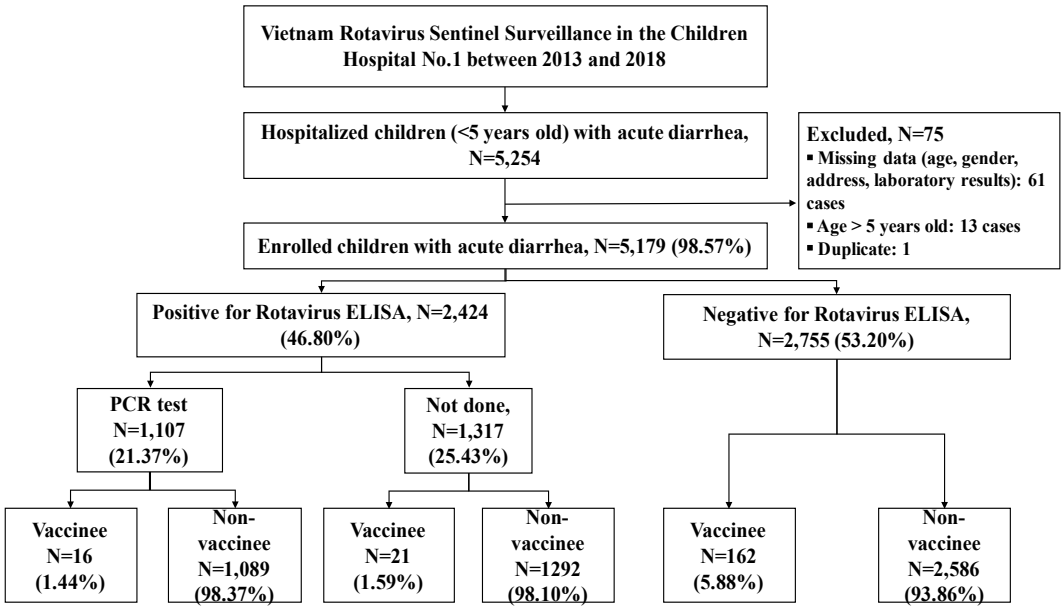

Figure 4.1. Flow diagram of enrollment of diarrhea cases in the sentinel surveillance, Southern Vietnam, 2013-2018.

Table 4.1. Characteristics of the study population, 2013-2018.

Characteristics	Total		Rota-positive AGE		Rota-negative AGE		p-value
	N=5,179		N=2,424		N=2,758		
	n	(%)	n	(%)	n	(%)	
Age							
Average age	13.03±8.42		14.03±8.48		12.15±8.27		<0.001[a***]
< 6 months	572	11.04	178	7.34	394	14.30	<0.001[b***]
6-11 months	2,169	41.88	940	38.78	1,229	44.61	
12-23 months	1,914	36.96	1,019	42.04	895	32.49	
24-59 months	524	10.12	287	11.84	237	8.60	
Gender							
Male	3,317	64.05	1,554	64.11	1,763	63.99	0.93[b]
Female	1,862	35.95	870	35.89	992	36.01	
Geographic distributions							
South-eastern region	2,812	54.30	1,242	51.24	1,570	56.99	<0.001[b***]
South-western region	1,852	35.76	930	38.37	922	33.47	
Other region	515	9.94	252	10.40	263	9.55	
Years							
2013	825	15.93	456	18.81	369	13.39	<0.001[b***]
2014	842	16.26	388	16.01	454	16.48	
2015	827	15.97	374	15.43	453	16.44	
2016	914	17.65	417	17.20	497	18.04	
2017	958	18.50	435	17.95	523	18.98	
2018	813	15.70	354	14.60	459	16.66	

Characteristics	Total		Rota-positive AGE		Rota-negative AGE		p-value
	N=5,179		N=2,424		N=2,758		
	n	(%)	n	(%)	n	(%)	
Month							
January	360	6.95	253	10.44	107	3.88	<0.001[b***]
February	334	6.45	241	9.94	93	3.38	
March	441	8.52	272	11.22	169	6.13	
April	393	7.59	177	7.30	216	7.84	
May	474	9.15	187	7.71	287	10.42	
June	376	7.26	93	3.84	283	10.27	
July	362	6.99	121	4.99	241	8.75	
August	499	9.64	175	7.22	324	11.76	
September	403	7.78	161	6.64	242	8.78	
October	444	8.57	177	7.30	267	9.69	
November	601	11.60	287	11.84	314	11.40	
December	492	9.50	280	11.55	212	7.70	

[a] ANOVA; [b] Chi-square test
[*] p<0.05; [**] p<0.01; [***] p<0.001

4.1.1. Age distribution

As is presented in Table 4.1, the mean admission age of all hospitalized children in the study was 13.02±8.42 months. Rotavirus positive group was significantly older than the Rotavirus negative group (14.03±8.48 versus 12.15±8.27, p<0.001).

Table 4.1 also shows well over three quarters (78.84%) of the study population were from 6 to 23 months old. There was a significantly different in the Rotavirus positive rate between four age groups; children older than 23 months of age had the highest proportion of Rotavirus gastroenteritis (54.77%), while group 0–5 months had the lowest proportion (31.12%) (p<0.001).

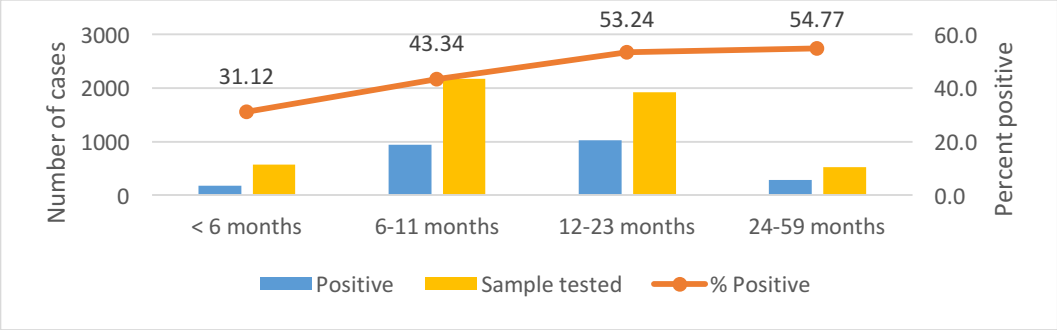

Figure 4.2. Age distribution of children admitted with acute diarrhea, Rotavirus positive cases, and percent positive, 2013–2018.

4.1.2. Gender distribution

Of the 5,179 fecal samples, males accounted for a higher percentage of acute diarrhea than females (64.05% compared to 35.95%) and Rotavirus positive cases (61.11% versus 35.89%), but it was not statistically significant ($p>0.05$).

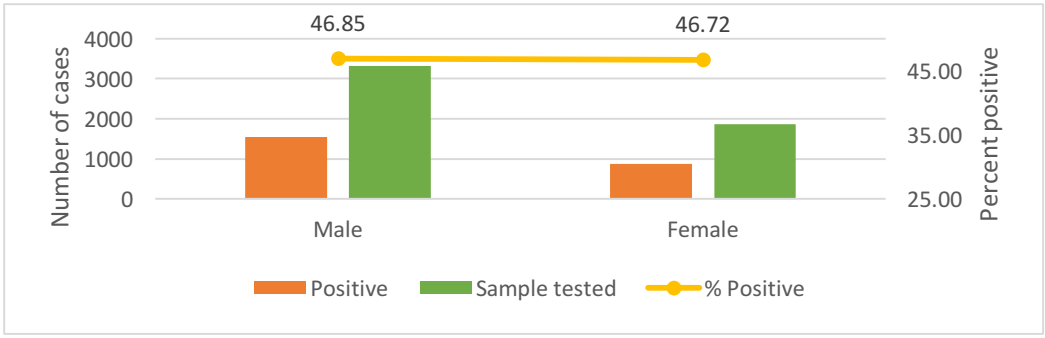

Figure 4.3. Gender distribution total sample tested, Rotavirus positive cases, and percent positive, 2013–2018.

4.1.3. Geographic distribution

There was significantly different in the prevalence of Rotavirus in different regions ($p<0.001$). Compared to other regions, the South-eastern region had the highest hospitalized diarrhea cases (2,812 cases, constituted 54.30%); however, it had the lowest positive Rotavirus rate among sample tested (44.17%). South-western region only accounted for 35.76% of the study population, but it had the highest positive rate (50.22%).

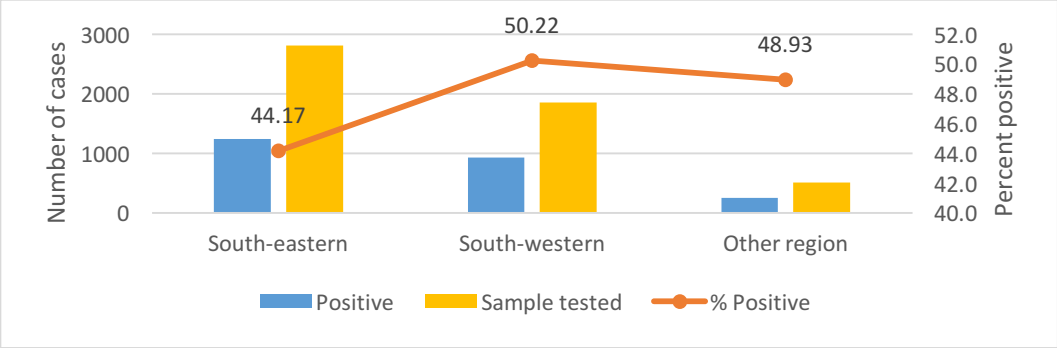

Figure 4.4. Geographic distribution of total sample tested, Rotavirus positive cases, and percent positive, 2013–2018.

4.1.4. Yearly distribution

Among all study population, there was a significant difference in the prevalence of Rotavirus in different years ($p<0.001$). A downward trend was observed in the Rotavirus positive rate from 2013 (55.27%) to 2018 (43.54%) (Figure 4.5).

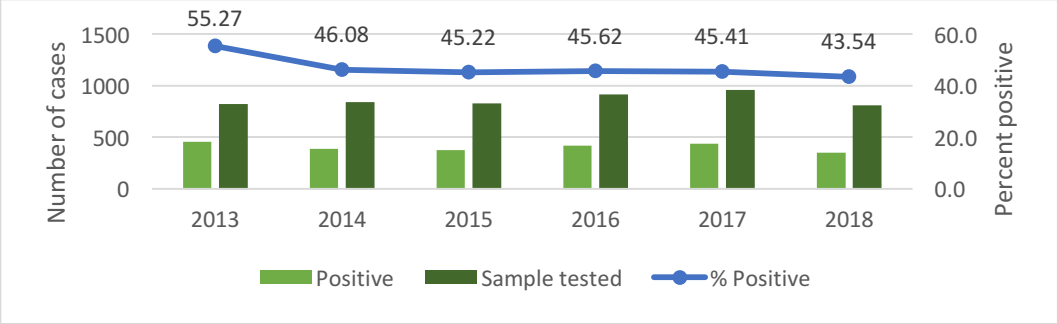

Figure 4.5. Yearly distribution of total sample tested, Rotavirus positive cases, and percent positive, 2013-2018.

4.1.5. Monthly distribution and seasonality

In terms of cumulative monthly distribution, the Rotavirus positive rate ranged from a maximum of 71.26% in February to a minimum of 24.73% in June (Figure 4.6).

Acute diarrhea and Rotavirus infection were detected year-round; however, it can be seen that Rotavirus positivity varied seasonally (Figure 4.6 and 4.7). Dry season months

(from November to April) witnessed a higher occurrence of Rotavirus positive cases (62.29%, 1,510/2,424 cases) compared to rainy season months (May to October, 37.71%, 914/2,424 cases), and this difference was statistically significant (p<0.001).

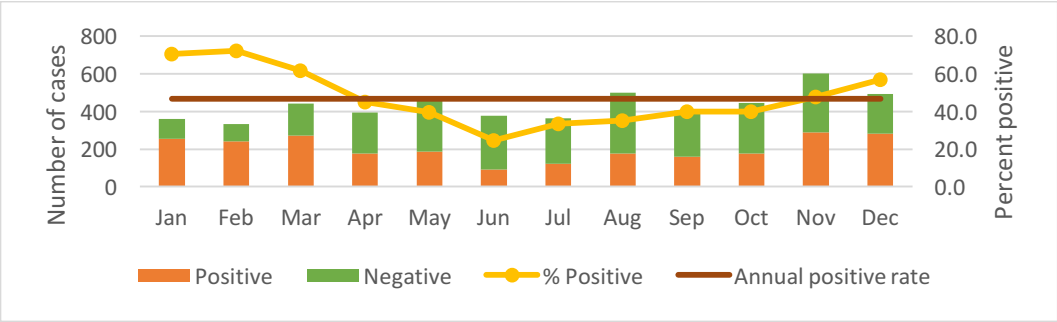

Figure 4.6. Cumulative monthly distribution Rotavirus positive and negative cases of acute diarrhea, 2013-2018.

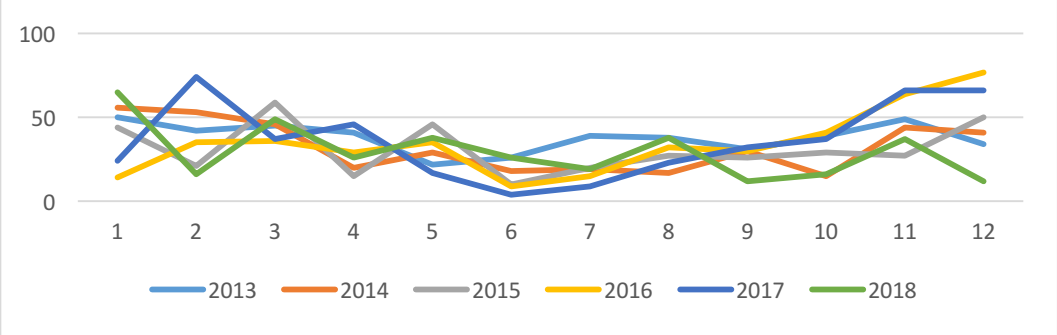

Figure 4.7. Monthly distribution of Rotavirus AGE positivity from 2013 to 2018.

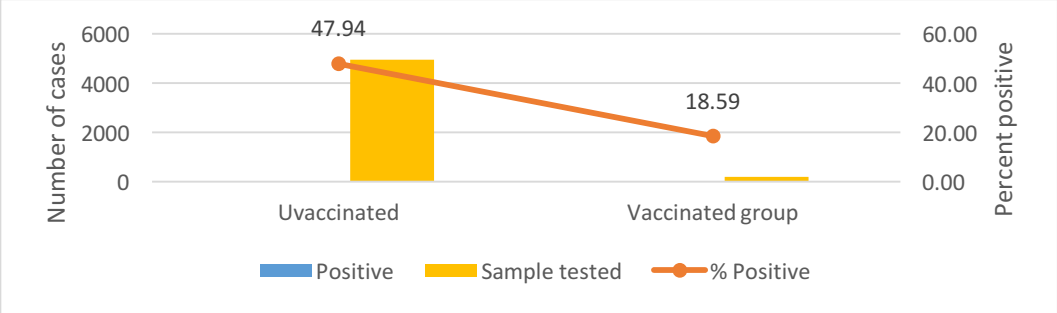

Figure 4.8. Distribution of vaccination history in terms of total sample tested, Rotavirus positive cases, and percent positive, 2013-2018.

4.1.6. Vaccination history

The percentage of vaccinees among the study population was meager at 3.84%. The positive rate in the vaccinated group was 18.59%, whereas in the unvaccinated group was 47.94%, the difference was statistically significant (p<0.001) (Figure 4.8).

The majority of vaccinees took the Rotarix (67.34%, 134/199), in which 17 cases were positive with Rotavirus. There was a significantly difference in the Rotavirus positivity of different vaccine names (p<0.05) (Table 4.2).

Table 4.2. Rotavirus vaccination history before infection, 2013-2018.

Characteristics	Total		Rota-positive AGE		Rota-negative AGE		p-value
	n	(%)	n	(%)	n	(%)	
Rota vaccination history before infection (n=5,179)							
Unvaccinated	4,967	95.91	2,381	98.23	2586	93.87	<0.001[b***]
Completed	130	2.51	18	0.74	112	4.07	
Incompleted	40	0.77	11	0.45	29	1.05	
Unknown vaccine name or dose	29	0.56	8	0.33	21	0.76	
Unknown vaccine history	13	0.25	6	0.25	7	0.25	
Vaccine names (n=199)							
Rotarix	134	67.34	17	45.95	117	72.22	0.012[b*]
Rotateq	27	13.57	10	27.03	17	10.49	
Rotavin	10	5.03	2	5.41	8	4.94	
Unknown vaccine name or dose	28	14.07	8	21.62	20	12.35	

[a] ANOVA; [b] Chi-square test
[*] p<0.05; [**] p<0.01; [***] p<0.001

4.1.7. Clinical features of participants according to Rotavirus results, 2013-2018

4.1.7.1. Clinical features

Of 5,007 participants that had vomiting data, 2.914 (58.20%) presented vomiting. Vomiting symptom was significantly more common in rota-positive than in rota-negative AGE (64.31% compared to 52.92%, $p<0.001$). The average maximum number of vomiting per day and duration of vomiting in rota-positive AGE patients were fractionally higher than in those who had negative results ($p<0.001$).

Of 3,512 hospitalized children who had data on fever symptoms, 1,105 cases (31.46%) had a fever. 33.46% (530/1,580) of Rotavirus AGE presented fever, while its percentage was considerably lower in negative cases (29.76%) ($p<0.05$). The difference between the average max temperature of rota-positive AGE and negative-AGE was not statistically significant ($p>0.05$).

Most patients did not present dehydration (95.00%). There was a significantly different in dehydration status between rota-positive and negative patients ($p<0.05$).

The average max number of diarrhea per day evaluated in 5,136 participants was 7.92±3.14 days. The average number in rota-positive cases was slightly higher than in rota-negative cases (8.06±3.15 compared to 7.80±3.13 days); the difference was statistically significant ($p<0.01$). The duration of diarrhea of the rota-positive AGE was shorter than the rota-negative AGE, but it was not statistically different (3.06±2.27 days compared to 3.13±2.36 days, $p>0.05$).

In the Vesikari scale, 44.73% of the Rota-positive AGE was severe, while its prevalence in the negative group was 32.76%. There was significantly different in the clinical severity between rota-positive and negative patients ($p<0.001$).

4.1.7.2. Treatments

The majority of hospitalized children was treated with ORS; the different between rota-positive and negative group was not statistically significant ($p>0.05$).

Of 438 children required IV treatment, children with Rotavirus-positive AGE were more likely to require IV than children with Rotavirus-negative AGE ($p<0.01$).

Of 441 children treated with antibiotics, children with rota-positive AGE were less likely to use antibiotics than the negative group; the difference was statistically significant ($p<0.01$).

Probiotic was given to nearly one-fourth of the patients; there was no statistical difference between the percentage of probiotic treatment in different Rotavirus results ($p>0.05$).

The average of hospitalization days for all patients from 2013-2018 was 4.45±3.96 days. The hospitalization duration of Rotavirus positive children was shorter than Rotavirus-negative AGE (4.25±3.69 versus 4.63±4.18 days). The difference was statistically significant ($p<0.01$).

4.1.7.3. Mortality

Of 5,083 cases who had data on the status of discharge, there was a Rotavirus associated death reported. The five-month-old child admitted to hospital in February of 2017 and was dead after eight days of hospitalization. The duration of diarrhea was two days, maximum number of diarrhea was seven times per day. He had vomiting for three times in one day, and no dehydration symptom. He also presented a fever with the highest temperature was 38-degree Celsius. The case was not randomized chosen for PCR genotyping, so we could not specify the genotype information.

Table 4.3. Clinical characteristics of participants, 2013-2018.

Characteristics	Total		Rota-positive AGE		Rota-negative AGE		p-value
	n	%	n	%	n	%	
Clinical features							
Vomitting (n=5,007)							
Yes	2,914	58.20	1,492	64.31	1,422	52.92	<0.001[b***]
None	2,093	41.80	828	35.69	1,265	47.08	
Average max. number of vomiting per day (n = 5,136)	4,956	3.14 ±3.60	2,299	3.63 ±3.70	2,657	2.72 ±3.45	<0.001[a***]
Duration of vomitting (n=4,299)	4,905	2.41± 2.98	2,266	2.64 ±2.96	2,639	2.21 ±2.98	<0.001[a***]
Dehydration (n = 5,120)							
none	4,864	95.00	2261	94.29	2,603	95.63	0.011[b*]
mild-moderate	239	4.67	132	5.50	107	3.93	
severe	17	0.33	5	0.21	12	0.44	
Fever (n = 3,512)							
Yes	1,105	31.46	530	33.54	575	29.76	0.016[b*]
No	2,407	68.54	1,050	66.46	1,357	70.24	
Average max temperature	1,279	38.34 ±0.76	610	38.32 ±0.74	669	38.35 ±0.76	0.40[a]
Average max. number of diarrhea per day	5,136	7.92 ±3.14	2,405	8.06 ±3.15	2,731	7.80 ±3.13	0.004[a**]
Duration of diarrhea	4,299	3.10± 2.32	2,012	3.06 ±2.27	2,287	3.13±2.36	0.30[a]

Characteristics	Total		Rota-positive AGE		Rota-negative AGE		p-value
	n	%	n	%	n	%	
Vesikari score (n = 3,356)							
< 7 (mild)	972	28.96	386	25.58	586	31.73	<0.001[b***]
7-10 (moderate)	1,104	32.90	448	29.69	656	35.52	
≥11 (severe)	1,280	38.14	675	44.73	605	32.76	
Treatment							
ORS (n = 5,159)	5,012	97.15	2,353	97.43	2,659	96.90	0.25[a]
IV (n = 5159)	438	8.49	236	9.77	202	7.36	0.002[b**]
Antibiotics (n = 5,159)	441	8.55	169	7.00	272	9.91	0.001[b**]
Probiotics (n = 3,414)	817	23.93	359	22.66	458	23.73	0.46[b]
Outcome							
Duration of hopitalization	4,989	4.45± 3.96	2,330	4.24±3. 68	2,659	4.63±4.19	<0.001[a**]
Mortality (n=5,083)	1	0.02	1	0.02	0	0	

[a] *ANOVA;* [b] *Chi-square test*
[*] *p<0.05;* [**] *p<0.01;* [***] *p<0.001*
There was some missing data according to each variables.

4.2. CHARACTERISTICS OF PATIENTS WITH ROTAVIRUS GASTROENTERITIS ACCORDING TO VACCINATION STATUS

Of 2,418 Rotavirus AGE children who had data on the history of vaccination, a small proportion was vaccinated (1.53%). There were no statistically significant in the prevalence of vaccine status in different groups of age, years, months, clinical features, and outcome ($p<0.05$).

The different between vaccination status in different geographic regions and different gender groups was statistically significant ($p<0.05$).

Table 4.4. Characteristics of patients with Rotavirus gastroenteritis according to vaccination status, 2013-2018.

Characteristics	Total		Vaccine group		Non-vaccine group		p-value
	n	%	n	%	n	%	
Age (n=2,418)							
Average	14.01±8.44		13.11±5.79		14.02±8.47		0.514[a]
< 6 months	177	7.32	3	8.11	174	7.31	0.17[b]
6-11 months	939	38.83	12	32.43	927	38.93	
12-23 months	1,017	42.06	21	56.76	996	41.83	
24-59 months	285	11.79	1	2.7	284	11.93	
Gender (n = 2,418)							
Male	1,549	64.06	18	48.65	1,531	64.3	0.049[b*]
Female	869	35.98	19	51.35	850	35.7	
Years (n = 2,418)							
2013	456	18.88	12	32.43	444	18.65	0.237[b]
2014	386	15.98	7	18.92	379	15.92	
2015	373	15.45	6	16.22	367	15.41	
2016	416	17.23	5	13.51	411	17.26	
2017	434	17.97	5	13.51	429	18.02	
2018	353	14.62	2	5.41	351	14.74	
Month (n = 2,418)							
January	253	10.48	2	5.41	251	10.54	0.603[b]
February	241	9.98	4	10.81	237	9.95	
March	271	11.22	2	5.41	269	11.3	
April	176	7.29	5	13.51	171	7.18	
May	187	7.74	5	13.51	182	7.64	
June	91	3.77	2	5.41	89	3.74	
July	120	4.97	2	5.41	118	4.96	

Characteristics	Total		Vaccine group		Non-vaccine group		p-value
	n	%	n	%	n	%	
August	175	7.25	3	8.11	172	7.22	
September	161	6.67	0	0	161	6.76	
October	177	7.33	4	10.81	173	7.27	
November	287	11.88	4	10.81	283	11.89	
December	279	11.55	4	10.81	275	11.55	
Geography (n = 2,418)							
South-eastern	1,239	51.30	27	72.97	1,212	50.9	0.028[b*]
South-western	927	38.39	8	21.62	919	38.6	
Other region	252	10.43	2	5.41	250	10.5	
Clinical features (##)							
Average max. number of vomiting per day	2,295	3.63±3.70	34	3.41±3.64	2,261	3.64±3.71	0.731[a]
Duration of vomitting	2,262	2.64±2.96	33	2.88±3.04	2,229	2.64±2.96	0.646[a]
Vomitting (n = 2,316)							0.959[b]
Yes	1,490	64.34	22	64.71	1,468	64.33	
None	826	35.66	12	35.29	814	35.67	
Dehydration (n = 2,394)							
none	2,258	94.32	36	97.3	2,222	94.27	0.725[b]
mild-moderate	131	5.47	1	2.7	130	5.52	
severe	5	0.21	0	0	5	0.21	

Characteristics	Total		Vaccine group		Non-vaccine group		p-value
	n	%	n	%	n	%	
Fever (n = 1,576)							
Yes	527	33.44	7	38.89	520	33.38	0.622[b]
No	1,049	66.56	11	61.11	1,038	66.62	
Average max temperature	607	38.32±0.74	10	37.89±0.78	597	38.33±0.74	0.062[a]
Average max. number of diarrhea per day	2,401	8.06±3.15	37	8.51±3.72	2,364	8.05±3.14	0.384[a]
Diarrhea duration	2,008	3.06±2.27	30	3.50±2.73	1,978	3.05±2.26	0.283[a]
Vesikari score (n = 1,505)							
< 7 (mild)	386	25.65	4	23.53	382	25.67	0.876[b]
7-10 (moderate)	446	29.63	6	35.29	440	29.57	
≥11 (severe)	673	44.72	7	41.18	666	44.76	
Treatment (##)							
ORS (n = 2,408)	2,349	97.55	36	100	2,313	97.39	0.326[b]
IV (n= 2,408)	236	9.80	5	13.89	231	9.73	0.406[b]
Antibiotics (n = 2,408)	169	7.02	3	8.33	166	6.99	0.748[b]
Probiotics (n = 1,580)	358	22.66	2	11.11	356	22.79	0.239[b]
Outcome (##)							
Duration of hopitalization	2,327	4.24±3.69	34	4.85±5.42	2,293	4.24±3.65	0.335[a]
Mortality (n=2,376)	1	0.04	0	0	1	0.04	

[a] ANOVA; [b] Chi-square test
[*] p<0.05; [**] p<0.01; [***] p<0.001
(##) There was some missing data.

4.3. GENOTYPE DISTRIBUTIONS

Table 4.5. Distribution of G and P genotypes among Rotavirus AGE, 2013-2018.

G type	P type										Total	
	P[4]	P[6]	P[8]	P [9]	P [11]	P [19]	P [Nt]	P[4] / P[8]	P[6] / P[8]	P[4]/ P [10]	n	%
G1	12	3	143	0	0	0	0	1	0	0	159	14.36
G2	142	0	20	0	0	0	0	2	0	0	164	14.81
G3	9	2	478	5	1	0	2	0	0	0	497	44.90
G4	0	3	2	0	0	0	0	0	0	0	5	0.45
G5	0	0	0	0	0	1	0	0	0	0	1	0.09
G8	1	1	218	0	0	0	0	1	0	1	222	20.05
G9	1	2	38	0	0	0	1	1	0	0	43	3.88
GNt	0	0	3	0	0	0	0	0	0	0	3	0.27
G1/ G3	2	0	9	0	0	0	0	0	1	0	12	1.08
G3/ G4	0	0	1	0	0	0	0	0	0	0	1	0.09
Total	167	11	912	5	1	1	3	5	1	1	1107	100
Total (%)	15.09	0.99	82.38	0.45	0.09	0.09	0.27	0.45	0.09	0.09	100	

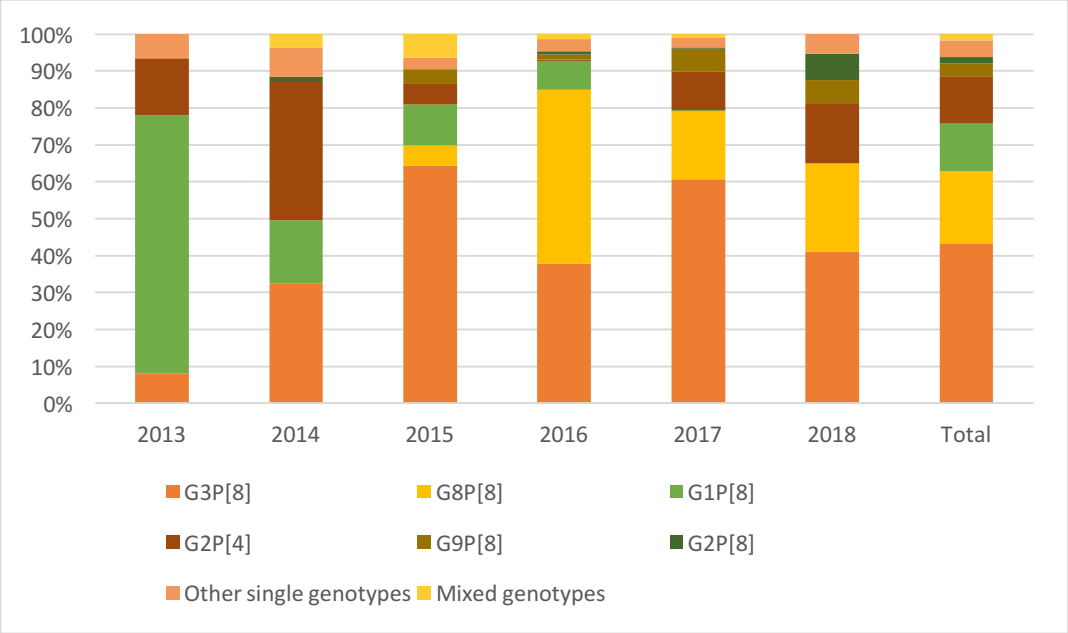

Figure 4.9. Genotypes distribution from Rotavirus positive cases isolated by PCR in sentinel surveillance, 2013-2018.

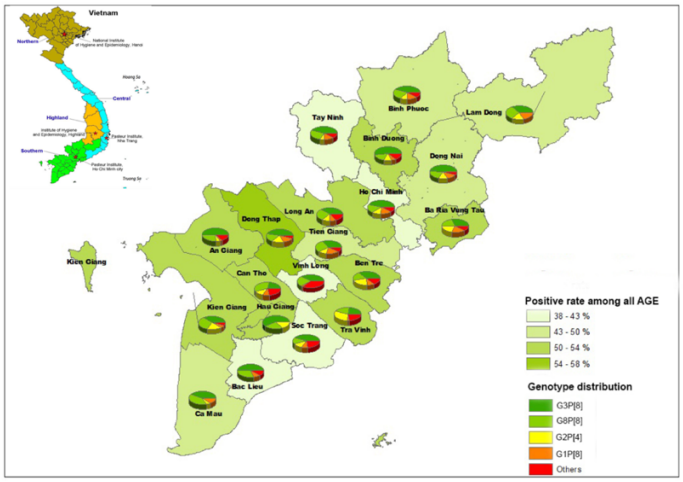

Figure 4.10. Positive rate among all AGE and genotype distribution according to geographic distribution in Southern Vietnam, 2013-2018.

Overall, of 1,107 Rotavirus positive cases had PCR isolated for genotyping.

G3 was the predominant G genotype, accounted for 44.90% (497/1,107). P[8] was the most frequently P genotype, constituted 82.38% (912/1,1107) (Table 4.5).

G3P[8] was the most common G-P combined genotype (43.18%), followed by G8P[8], G1P[8], G2P[4] (constituted 19.69%, 12.92%, and 12.83%, respectively).

Regarding G-P genotype combination according to year distribution, we observed an enormous shift from 2013 to 2018 (Figure 4.9):

- There was a considerable decline of G1P[8] from 69.92% in 2013 to no cases in 2018.
- G3P[8] became more frequent during 2014-2018, with the rise from 8.13% in 2013 to a peak of 60.65% in 2017, then decreased to 41.15% in 2018.
- There also occurred a change in G8P[8] and G2P[4] prevalence. From no case in 2013, G8P[8] leaped to a peak of 47.21% in 2016 and was the predominant genotype at that year, before went down to 23.92% in 2018.
- G2P[4] showed a slight upward trend from 2013 to 2014 (from 15.45% to 37.41%), then dropped to 0.43% in 2016, before went up to 16.27% in 2018.

In terms of geographic distribution, G3P[8] was the highest prevalence in most provinces, except Can Tho. In Can Tho, 27.27% genotypes isolated was G8P[8], equal with G3P[8] (Figure 4.10).

4.4. RISK ANALYSIS FOR ROTAVIRUS GASTROENTERITIS AMONG CHILDREN UNDER FIVE YEARS OF AGE

Table 4.6. Risk analysis for Rotavirus AGE, 2013-2018 (n=5,166).

Variables	Crude				Adjusted			
	OR	95%CI		p	OR	95%CI		p
Age								
< 6 months	Ref.	-	-	-	Ref.	-	-	-
6-11 months	1.693	1.391	2.06	<0.001	1.678	1.369	2.058	<0.001***
12-23 months	2.52	2.066	3.073	<0.001	2.543	2.069	3.126	<0.001***
24-59 months	2.68	2.094	3.431	<0.001	2.568	1.988	3.317	<0.001***
Sex								
Male	Ref.	-	-	-	Ref.	-	-	-
Female	0.995	0.888	1.115	0.931	0.993	0.882	1.119	0.914
Visit year								
2013	Ref.	-	-	-	Ref.	-	-	-
2014	0.692	0.57	0.839	<0.001	0.655	0.535	0.801	<0.001***
2015	0.668	0.55	0.811	<0.001	0.659	0.538	0.807	<0.001***
2016	0.679	0.562	0.82	<0.001	0.655	0.537	0.798	<0.001***
2017	0.673	0.558	0.812	<0.001	0.588	0.483	0.716	<0.001***
2018	0.624	0.513	0.759	<0.001	0.549	0.447	0.674	<0.001***
Month								
Rainy season	Ref.	-	-	-	Ref.	-	-	-
Dry season	2.445	2.186	2.734	<0.001	2.4	2.14	2.692	<0.001***
Geographic distributions								
South-eastern	Ref.	-	-	-	Ref.	-	-	-
South-western	1.275	1.134	1.434	<0.001	1.285	1.135	1.455	<0.001***
Others	1.211	1.004	1.462	0.046	1.249	1.025	1.522	0.028*
Rotavirus vaccination								
Unvaccinated	Ref.	-	-	-	Ref.	-	-	-
Vaccinated (at lease one dose)	0.248	0.173	0.356	<0.001	0.273	0.188	0.396	<0.001***

*$p<0.05$; **$p<0.01$; ***$p<0.001$

It was discovered that Rotavirus AGE was more likely to be found in children older than six months; compared to the under six months group:

- The odds of Rotavirus AGE in the 6-11 months group was 1.68 times higher (OR=1.678, 95%CI: 1.369 - 2.058, p<0.001).
- The odds of Rotavirus AGE in the 12-23 months group was 2.54 times higher (OR=2.543, 95% CI: 2.069 - 3.126, p<0.001)
- The odds of Rotavirus AGE in the 24-59 months group was 2.57 times higher (OR=2.568, 95% CI: 1.988 - 3.317, p<0.001).

Compared to the South-eastern region, Rotavirus AGE was more likely to occur in the South-western (OR=1.285, 95% CI: 1.135 - 1.455, p<0.001) and other region (OR=1.249, 95% CI: 1.025 - 1.522, p<0.05).

Dry season months (from November to April) witnessed a 2.4 times higher occurrence of Rotavirus positive cases compared to rainy season months (May to October) (OR=2.4, 95% CI: 2.14 - 2.692, p<0.001).

Compared to the unvaccinated group, the vaccinated group (with at least one dose) was at lower risk for Rotavirus positive (OR=0.273, 95% CI: 0.188 - 0.396, p<0.001).

4.5. VACCINE EFFECTIVENESS

Among children ≥ 6 months of age who were age-eligible to have received a full schedule of the Rotavirus vaccine, getting a completed schedule of Rotavirus vaccine had vaccine effectiveness against Rotavirus AGE at 82.34% (95% CI: 70.33%-89.49%) (Table 4.7).

Considering Rotarix vaccine effectiveness, getting a completed schedule of Rotarix had vaccine effectiveness against Rotavirus AGE at 85.90% (95% CI: 74.10%-92.30%). Rotarix VE for G3P[8] was 88.35% (95%CI: 52.50%-97.14%).

Table 4.7. Vaccine effectiveness

Rotavirus vaccination	Completed schedule		Unvaccinated		VE	95%CI		p
	cases	control	cases	control		-	-	
All vaccine								
VE	17	97	2,207	2,224	82.34	70.33	89.49	<0.001***
G1P[8]	1	97	131	2,224	82.50	-26.50	97.58	0.08
G2P[4]	2	97	133	2,224	65.52	-41.36	91.59	0.14
G3P[8]	2	97	444	2,224	89.67	57.96	97.46	0.002**
G8P[8]	2	97	202	2,224	77.30	7.25	94.44	0.039*
Rotarix only								
VE	12	86	2,207	2,224	85.90	74.10	92.30	<0.001***
G1P[8]	0	86	131	2,224	100	-	100	0.08
G2P[4]	2	86	133	2,224	61.11	-59.73	90.53	0.19
G3P[8]	2	86	444	2,224	88.35	52.50	97.14	0.003**
G8P[8]	2	86	202	2,224	74.40	-4.80	93.74	0.06

*$p<0.05$; **$p<0.01$; ***$p<0.001$

CHAPTER 5:

DISCUSSION

This retrospective study presented a 7-year result of the Rotavirus sentinel surveillance in Southern Vietnam from 2013 to 2018.

Several Rotavirus-associated diarrhea research had been performed across the country [4, 6, 62, 68, 72, 77-92]; however, most studies only focus on epidemiological and genotyping features. Our study not only provided an updated picture of Rotavirus epidemiology and genotypes but also discovered the clinical features and vaccination status among study population when the Rotavirus vaccine is self-financed in Vietnam.

5.1. CHARACTERISTIC OF STUDY POPULATION ACCORDING TO ROTAVIRUS ELISA RESULTS, 2013-2018

Of 5,179 acute diarrhea cases included in the study, Rotavirus was detected in 2,424 cases (46.80%). This prevalence was in harmony with the previous results in Vietnam during 2012-2015: the National Sentinel Surveillance report (46.66%, 4,054/8,689 cases) [4], as well as a study in five provincial hospitals across the country (50.2%, 678/1,350 cases) [72]. Our result was considerably more elevated than the global median positive proportion in 2013 (37.3%) and lower than the Rotavirus detection rates of Southeast Asia (50.7%–54.6%) [2].

Compare to other recent study with the same methods, our positive rate was higher than in other ASEAN countries, such as: Philippines (34%, 2014-2017) [93], Indonesia (31.7%, 2015-2018) [56]; and other Asian countries, such as: China (20.8%, 2011-2016) [94], Korea (32.9%, 2012-2013) [95], India (45%, 2014-2016) [96]. It was slightly lower than in Cambodia (50%, 2010-2016) [97], and Myanmar (49.90%, 2009-2014) [98]. The high Rotavirus positivity in Vietnam could be because of the weather characteristic, level of socio-economic population, and other reasons that will be discussed afterward.

5.1.1. Age distribution

The mean age of rota-positive AGE in this study was higher than the National Sentinel Surveillance data during 2012-2015 (13.7 months, 95% CI: 13.4–14.0) [4]. Our result was higher than the study in India (13.32 ± 9.55 months, between July 2014 and June 2016) [96], and less than in Korea (19.8 ± 13.6 months, during 2012-2013) [95].

We observed that Rotavirus diarrhea was more likely to be found in children older than six months. Using the under six months old group as reference variable, the odds of Rotavirus positive in other groups were higher. A lower positivity rate among children less than six months of age is probable that most of them are less contact to the outside world, and considered to be partially protected from Rotavirus infections by maternal antibodies through the placenta [99]. However, passive antibody titers wane over time and no longer confer protection after six months, which may explain why most children were infected with Rotavirus between 6 and 23 months [99]. A low positivity of Rotavirus was found among children from 24 months old could also be explained by the natural immunity conferred by past exposures [55]. Children can be infected Rotavirus more than one time, with the first episode of Rotavirus AGE by the first year of life. Several cohort studies described that one event of Rotavirus infection had a protective efficacy, and recurrent episodes of Rotavirus disease were less severe than the first episode [1], [4].

The similarity occurred in other countries. In China, the Rotavirus-positive rate was most common among the 1–2 years old age group [94]. Molecular epidemiological analysis in Indonesia described that Rotavirus infection was significantly more prevalent in the 6- to 11-month age group than in the other age groups ($p<0.05$) [56].

Besides, the age shift also can happen after the Rotavirus vaccine introduction. A previous study based on Global Rotavirus Surveillance Network from 2008-2016 indicated that in the post-vaccine period, the proportion of Rotavirus gastroenteritis cases occurring in the 0–5-month age group and 6–11-month age group were dropped. In contrast, the incidence increased for both the 12–23-month and the 24–59-month age group. This trend also was discovered in Rwanda, the cumulative age distribution of Rotavirus AGE showed

a rightward shift, with 56% of Rotavirus gastroenteritis hospital admissions occurring among infants in the pre-vaccine period compared with 31% after Rotavirus vaccine introduction [63]. In a similar trend, there was a fall in the prevalence of Rotavirus cases occurring by 12 months of age in Bolivia, from 67% in the pre-vaccine period to 55% in the post-vaccine period [64]. It remains to be seen whether this shift in the proportion of Rotavirus AGE to older ages is a reflection of improved protection shortly after vaccination, or this shift will diminish over time as all cohorts up to five years of age are vaccinated, or the absolute number of Rotavirus gastroenteritis cases will change. Also, there might be differential enrollment practices by age between pre-vaccine and post-vaccine countries that would affect this age distribution; this would need further study [53].

5.1.2. Gender distribution

In our study, males accounted for a high percentage than females, but there was no statistically significant ($p>0.05$). A similar trend was observed in the study of Huyen et al. (64.6% male compared to 35.4% female) and the study of Chung et al. in Korea (the sex ratio of male: female was 1.23) [95].

5.1.3. Geographic distribution

The South-eastern region had the highest hospitalized diarrhea cases (54.30%); however, compared to South-eastern region, Rotavirus AGE was more likely to occur in South-western and other region.

This results may happen because the Children Hospital No. 1 in Ho Chi Minh city is located in the South-eastern region, so parents tended to take their child to the hospital if they appeared any symptoms. The South-western region is far from the hospital site, so parents tended to use the provincial hospitals or clinic services; only severe cases would be transferred or visit the Children Hospital No. 1.

5.1.4. Yearly distribution

A downward trend was observed in the Rotavirus positive rate from 2013 (55.27%) to 2018 (43.54%). A reduction also identified in the previous study in Vietnam [4]. There is a need to study further to identified the reason for this slip.

5.1.5. Monthly distribution and seasonality

The tropical climate in Southern Vietnam is characterized by high temperatures year-round and sunny weather. Mean annual temperatures in coastal areas are around 27°C that is fairly even throughout the year with little difference between the coldest and hottest months of the year. There are two seasons per year: rainy seasons from May to October, dry season from November to April.

In our study, acute diarrhea and Rotavirus infection were detected throughout the year; however, it can be seen that Rotavirus positivity varied seasonally. Dry season months (from November to April) witnessed a higher occurrence of Rotavirus positive cases compared to rainy season months (May to October) ($p<0.001$).

A similar trend was observed in other Southeast Asia countries, such as Thailand (the highest rate months each year were November, December, and January), and Cambodia (incidence peaks typically occurred between November-May) [94], [97]. By contrast, in Korea, a country is located in the temperate zone, Rotavirus detection peaked in March and April but rapidly decreased in May [95].

These results suggest that considerable attention to local climatic conditions may help our understanding of the prevalence of Rotavirus infection. Most studies discovered that the distinct winter seasonality of Rotavirus hospitalizations in temperate environment stands on the contrary to the year-round disease seen in tropical climates. It means that a child born in a temperate setting right after the Rotavirus season may have to wait many months before encountering the first possible natural infection in the following year's winter. On the contrary, a child born in a tropical climate may be exposed any day. Consequently, the average age at first infection is often younger in developing countries in tropical areas. In such tropical countries, the early episode of Rotavirus disease occurs most often during the first year of life, whereas frequently in developed countries, the highest prevalence of first Rotavirus infections occurs in the second year of life. Therefore, an effective vaccine program in a developing country may require earlier and higher levels of coverage than in a developed country [12].

5.1.6. Vaccination history

Vietnam did not set surveillance on private sector vaccine, so to date, there is no available data about Rotavirus coverage in the country. In this study, the percentage of vaccinees among hospitalized AGE children was extremely low (3.84%).

The Rotavirus vaccine in Vietnam is self-financed by caregivers. To get the vaccine in the private sector, the family should pay by themselves with a high price compared to Vietnamese income. That factor might be a reason for the low coverage of the Rotavirus vaccine in the country. Besides, other factors that should be considered are children whose mothers had relatively less household wealth, were from ethnic minorities, lived in rural areas, and had less education. At the community level, the child's region of residence was the main predictor of timely immunization completion, and the availability of hospital delivery and community prenatal care in the local community were also determinants [100].

Compared to the unvaccinated group, the vaccinated group was at lower risk for Rotavirus positive. A similar observation was described in Korea: the Rotavirus AGE incidence is lower in the Rotavirus-vaccinated group compared to the unvaccinated group with no evidence of substitution with unusual genotype combinations [95].

5.1.7. Clinical features of participants according to Rotavirus results, 2013-2018

5.1.7.1. Treatments

According to WHO, no specific therapy is currently available against Rotaviruses. As with other childhood diarrheas, the corner-stones of treatment are fluid replacement to prevent dehydration, and zinc treatment, which decreases the severity and duration of diarrhea. Additional treatment measures during the diarrheal episode include continued feeding, including breast-feeding, and if ORS is not available, use of appropriate fluids available in the home [1].

In this study, children with rota-positive AGE were less likely to use antibiotics than the negative group; the difference was statistically significant ($p<0.01$). The reason for this trend may because at the hospital, if the disease pathogen was not classified (Rotavirus negative), doctors tended to give antibiotics to treat a widen pathogen.

5.1.7.2. Outcomes

The hospitalization duration of Rotavirus positive children was shorter than Rotavirus-negative AGE (4.25±3.69 versus 4.63±4.18 days, $p<0.001$). The longer duration of hospitalization in Rotavirus negative group might happen because at the hospital, patients without an apparent disease pathogen tended to take a longer time to diagnosis and treatment. However, there still need more study to give evidence on the assumption.

Mortality: There was one Rotavirus associated death reported. The mortality in this study is extremely low compared to the global CFR of approximately 2.5% among children in developing countries who present to health facilities. The CFR is higher in areas without proper access to health care [52]. The death case in our study was not randomized chosen for PCR genotyping, so we couldn't specify the genotype information for further analysis, this issue revealed a need of considering to include specific important cases into the PCR for genotyping in the process of sentinel surveillance.

5.2. THE GENOTYPIC DIVERSITY OF ROTAVIRUS STRAINS DETECTED IN CHILDREN AGED <5 YEARS IN SOUTHERN VIETNAM, 2013-2018

Genotyping has an enormous value for assessing the evolution and epidemiological pathways of Rotavirus in humans, mammals, and birds [15]. Despite the broad genomic and antigenic diversity of Rotavirus, globally, only a small number of Rotavirus types have prevailed in humans during the past three decades. Currently, 5 G-P combinations (G1P[8], G2P[4], G3P[8], G4P[8]) and G9P[8]) account for approximately 90% of all human Rotavirus infections in many parts of the world; type G1P[8] is the most prevalent combination. However, data from countries in Asia and Africa show greater strain diversity with several Rotavirus types circulating simultaneously; other genotypes such as G5, G6, and G8 are more prevalent [1], [15].

In our study, of 1,107 Rotavirus positive cases had PCR isolated for genotyping; G3 was the predominant G genotype, P[8] was the most frequently P genotype. G3P[8] was the most common G-P combined genotype, followed by G8P[8], G1P[8], G2P[4].

We observed an enormous shift from 2013 to 2018: G3P[8] became predominant while G1P[8] considerably dropped. Globally, after the vaccine introduction, the prevalence of G1P[8] strains has significantly decreased [65], [101]. In recent years, G3P[8] Rotavirus has emerged as a predominant genotype in several countries with a wide range of vaccine coverage, including Asia (Pakistan, Indonesia, Japan, Thailand), South-America (Argentina), and Europe (Germany, Spain), Australia [56], [65], [102], [103]. The global emergence of G3P[8] strain happened irrespective of vaccine coverage, suggested that vaccines may not be highly effective against this strain.

We also discovered a change in G8P[8] and G2P[4] prevalence. G8P[8] also emerged after 2014 in Thailand and caused outbreaks in Central Japan in 2014 and 2017 [66]. G2P[4] strains had increased in circulation in many countries after the Rotarix vaccine introduction, such as Australia, Brazil, Belgium, and China [65], [104, 105]. This variability raised the question of whether the genotype changes were due to natural fluctuation of Rotavirus populations, or if the vaccines were less effective at protecting against certain strains.

It is difficult to determine the apparent cause of these genotyping changes. However, it is considered that Rotavirus diversity is generated by genetic drift, genetic shift, and zoonotic transmission [106]. Theoretically, any of the known P and G complexes could recombine to form new viruses, assuming these recombinations created sustainable viruses. Vaccine-induced immune pressure may be an additional selective pressure that leads to the evolution of Rotavirus strains capable of causing severe disease in vaccinated children [65]. Monitoring such changes is necessary to ensure the long-term safety of the Rotavirus vaccine.

5.3. VACCINE EFFECTIVENESS

Vietnam has licensed two WHO-prequalified vaccines and one national-licensed vaccine: Rotarix (GlaxoSmithKline, Rixensart, Belgium), which is based on the human Rotavirus G1P[8] strain; and RotaTeq (Merck and Company, Whitehouse Station, New Jersey, USA), consisting of five human-bovine recombinant strains. The Vietnamese local-

state Rotavin-M1 vaccine, which is based on the G1P[8] strain, is similar to the monovalent Rotarix vaccine, but the viral sequence and replication ability of the Rotavin-M1 vaccine isis different from the Rotarix strain [8]. Introduction of Rotavirus vaccination may be closely associated with the strain selection of Rotavirus genotypes [65] [66]. The study of Fischer et al. in 2003 found that universal Rotavirus vaccination in Viet Nam can prevent 70% of the outpatient visits, 84% of hospitalizations, and 83% of the related deaths [5].

Our study was conducted in the context of meager vaccine coverage. Among children ≥ 6 months of age who were age-eligible to have received a full schedule of Rotavirus vaccine, a 82.84% reduction against Rotavirus AGE was among children who got a full schedule of Rotavirus vaccine. Most of the vaccinated cases were used the Rotarix vaccine, so we did further analysis on the Rotarix VE. The VE of the completed Rotarix schedule (85.90%) was higher than overall VE.

Our VE result is considered to be a high VE compare to other studies. A systematic review of 48 articles from 2006-2016 with post-licensure data from 24 countries showed a median Rotarix VE of 84%, 75%, and 57% in countries with low, medium, and high child mortality, respectively, and Rotateq VE of 90% and 45% in countries with low and high child mortality, respectively [107]. By income, vaccine effectiveness was high in high-income countries with protection rates against severe Rotavirus disease at 80–90% [108], [109], [107]; it was 30–50% lower in low- and middle-income countries, mainly in sub-Saharan Africa and South East Asia [2], [110], [111], [112], [113], [107]. Several health conditions have been recognized as being important for the outcome of Rotavirus vaccine: malnutrition with deficiencies in micronutrients (zinc, vitamin A, vitamin D), connected with functional reduction of innate and acquired immune responses, and the gut microbiome which is of proven influence for disease severity and vaccine uptake. Maternal Rotavirus-specific antibodies are of variable importance for disease and vaccine outcomes. Specific diarrhea prevention programs (supply of nutrients and micronutrients, such as vitamins, combined with Rotavirus vaccination) have been shown to be beneficial [107].

Besides, Moon et al. hypothesized that the neutralizing effect of breast milk could play an essential role in reducing the antigen dose when breast milk is consumed immediately before vaccination [114]. However, several newer studies have not shown any beneficial effect of temporarily withholding breastfeeding for 30 to 60 minutes before and after vaccine administration on the immune response to the Rotavirus vaccine [115], [116]. In 2010, there was a study conducted in Vietnam examined possible differences in breast milk Rotavirus-specific antibody level, focusing on the level of neutralizing activity against the Rotavin-M1 vaccine strain. An increase in neutralizing anti-G1P[8] antibody titers ($P < 0.05$) in rural infants over time suggests a continuous exposure to circulating Rotavirus; breastfeeding could be hold off at the time of vaccination and resume one hour after vaccine delivery to enhance the immune responses in children [86].

To introduce a Rotavirus vaccine in Vietnam, the strain diversity will be a challenge; G1P[8] used to be common but was declined, while G3P[8] Rotavirus has emerged as a predominant genotype. In this study, because the number of cases in G1P[8] was low, so we could not calculate the Rotarix VE this genotype; however, we identified a noticeable result that Rotarix VE for G3P[8] was high at 88.35% (95%CI: 52.50%-97.14%). This result may reveal the cross-serotype protection provided by the Rotarix vaccine in Southern Vietnam, which will be a considerable reason while consider to use the Rotavirus monovalent vaccine in the country.

The ability of Rotavirus monovalent vaccine to generate broader heterotypic protection was estimated in a systematic review in 2014: Rotavirus monovalent vaccine provided broad clinical efficacy and field effectiveness against severe diarrhea due to all major circulating strains, including the homotypic G1P[8], partially heterotypic (G3P[8], G4P[8], and G9P[8]), and fully heterotypic G2P[4] Rotavirus strains throughout different geographical and income settings. The pooled effectiveness of monovalent Rotavirus vaccine in these high and middle-income countries was 77% (95% CI: 58%-86%) against homotypic strains, 72% (95% CI: 49%-84%) against partially heterotypic strains, and 67% (95% CI: 52%-77%) against fully heterotypic strains [117].

The cross-serotype protection mechanisms of Rotarix are still incompletely understood but likely are multi-factorial. First, Rotavirus immunity is polygenic as it induces neutralizing antibodies to VP7 and VP4 and non-neutralizing antibodies to other structural viral proteins VP1, VP2 and VP6, and to nonstructural viral proteins NSP2, NSP5, and the viral enterotoxin NSP4. Each viral protein-specific antibody might play a protective role. Second, different genotypes of Rotavirus share neutralization epitopes on VP4 and VP7, which could induce cross-reactive neutralizing antibody and protection. Besides, other mechanisms, such as T cell-mediated immunity or other host factors, may contribute to cross-protection [117].

5.4. LIMITATIONS OF THE STUDY

Our study was just included the sentinel surveillance in Southern Vietnam, which may not represent the whole country. All of the pediatric patients with AGE in this study were hospitalized, suggesting that their symptoms were more severe than those of non-hospitalized cases. Since we did not collect samples from non-hospitalized children, it is difficult to draw any conclusion about the severity of Rotavirus-associated AGE among children in the community. Further studies involving both hospitalized and non-hospitalized cases of Rotavirus gastroenteritis are needed.

5.5. FURTHER RESEARCH

Regarding the overall prevalence estimates of particular Rotavirus genotypes, the problem of sampling bias should not be underrated. The Rotarix VE against G3P[8] predominant genotype was considerable high; however, with the dramatic genotyping shift being identified in this study, there is still necessary to consider the strains of the recommended vaccine, which will be used in the National Immunization Program in the near future. Continued surveillance is needed to monitor changes in Rotavirus epidemiology before and after vaccine introduction. In addition to monitoring genotypes, whole genomic characterization of circulating Rotavirus strains will help assess whether vaccines are affecting the evolution of the Rotavirus genome.

CHAPTER 6:

CONCLUSION

Our study suggested that Rotavirus infection was still a significant cause of acute watery diarrhea among hospitalized children younger than five years old in Vietnam. Rotavirus positivity varied seasonally with the peak rates during the dry season of November to April.

Rotavirus AGE was more likely to be found in children ≥ 6 months. In terms of clinical features, it was more severe and less likely to be treated with antibiotics than the negative group.

We also observed an enormous genotyping shift from 2013 to 2018: G3P[8] became predominant while G1P[8] considerably dropped. There is a need to consider the recommended vaccine to use in the National Immunization Program within the dramatic genotyping shift situation.

In the context of low vaccine proportion, among children ≥ 6 months, getting a full schedule of Rotavirus vaccine in general and Rotarix, in particular, had vaccine effectiveness against Rotavirus AGE at 82.34% and 85.90%, respectively. Noticeably, Rotarix VE for G3P[8] was 88.35% (95%CI: 52.50%-97.14%). Whole genomic characterization of circulating Rotavirus strains before and after vaccine introduction will help to assess the vaccines' efficacy.

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Appendix 1

ROTAVIRUS REPORTING FORM

Form 27/2012-TCMR

Suspected case: A child 0-59 months has acute (<14 days) watery diarrhea, defined as three or more in a 24-hour period.

PART I. FOR HOSPITAL

A. General information

1. Case ID: | | | | | | | | | |
2. Hospital name: ……..……..…….
3. Medical record number: …… Date of hospital amidssion: ……./……./…
4. Patient's name: ……Gender: □ Male □ Female
5. Date of birth: _____/___/_____
6. If date of birth is unknown, age of the child: ..... years old, or …… months old, or …… days old.
7. Address: Number/ Village……Ward/ Commune:…… District:…… Province:……
8. Contact number: ……

B. History and treatment

9. Did the child vaccinated Rotavirus vaccine? □ Yes □ No
10. Date of vaccinated: 1st doset___/___/___2nd dose___/____/___ 3rd dose___/____/____
11. Source of information: □ Immunization card □ Asking □ Unknown
12. Had diarrhea in: …… days. Maximum number of diarrhea in a 24-hour period:…….
13. Had vomitingin: ……days. Maximum number of vomitted in a 24-hour period: …….
14. Dehydration level: □ No □ Some dehydration □ Severe dehydration

15. Had treatment: □ ORS □ IV □ Antibiotic
    □ Probiotic □ Others: ……... □ Unknown
16. Chil point of entry: □ Emergency □ OPD □ Gastroenterology □ Others
17. Status of discharge: □ Alive □ Dead (date of daed:___/___/_____) □ Unknown
    Date of discharge:___/___/__
18. Stool sample collected: □ Yes □ No □ Unknown
    If yes, date of stool sample collected:_____/_____/_____
19. Other comments: ……

Date of report……/…../……

Investgator

(Name, Signiture)

PART II. FOR PASTEUR INSTITUTE

1. Laboratory number: ……
2. Date of stool sample received: ____/____/_____
3. Stool characteristic: □ Watery □ Non watery □ Bloody □ Other.....
4. Stool codition: □ Good (No spillage, cold) □ Warm when touch the sample □ Broken
5. Stool quantity: □ Sufficient □ Unsufficient □ Other.....
6. ELISA (EIA) resul: □ Positive □ Negative □ Suspected □ Didn't make the test
   Date of having result:____/___/_____ Date of reporting result:____/___/_____
7. Genotyping: G type ___ P type ___ Unknown: □ G type □ P type □ Both G & P
   Date of having genotyping result:___/___/___
   Date of reporting genotyping result:___/___/___
8. Other comments: ……

Laboratory

(Name, Signiture)